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HISTOCHEMICAL REACTIONS IN CARCINOID TUMORS OF THE HUMAN GASTROINTESTINAL TRACT

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The older literature on the structure and clinical behavior of carcinoid tumors has been adequately reviewed by Oberndorfer.[1,2] It was he who clearly separated them from carcinoma and first applied the name carcinoid to them, and Masson[3] who first described their property of argentaffinity.[4,5] Feyrter,[6] among others, also reviewed the subject. The recognition of the chromaffin reaction of these lesions is ascribed to Oberndorfer.[7] Since this reaction practically requires primary chromate fixation and is uncertain even on normal mammalian intestinal material, it has been almost completely supplanted by Masson's argentaffin reaction.[4,7-9] This was reported first in 1914 as successful on tissue with formaldehyde and Bouin fixations, or by some other silver reduction technique.

Pearse[10] states that there are two schools regarding the diagnosis of carcinoid tumors. One considers the presence of a positive silver reduction as adequate confirmation of the morphologic diagnosis. The other, to which he adheres, requires additional positive reactions, such as acid and alkaline azo coupling and the Gibbs reaction. Unfortunately, neither of these schools has prevailed. Instead, the attitude has developed that the diagnosis of carcinoid is readily made on morphologic grounds alone. Of 922 cases encountered in a partial coverage of the literature, 739 or over 80 per cent must be placed in this class (Table I). Many of the authors whose cases fall into this class mention the argentaffin reaction

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in their discussions, but make no statement indicating that they used it in their cases. Others report a few cases in detail in which one or another silver reaction was used, and then tabulate a larger number of cases in which no statement is made about silver reactions. This attitude was supported by Feyrter's statement [6] that all carcinoids are silver positive when the material is obtained surgically or within 5 to 6 hours post mortem and promptly fixed in a proper fixative.

Since it now appears that the presence or absence of reduction, azo or other specific reactions may correlate with the functional status of the carcinoid, the argentaffin reaction cited in the past literature acquires further significance beyond that of mere confirmation of morphologic recognition. The structure of the typical carcinoid of the appendix, small intestine and colon has been well characterized by Oberndorfer, Masson, Feyrter and many others. Suggestions exist that gastric and rectal carcinoids may constitute separate varieties, which differ in some morphologic particulars and in infrequency of a positive argentaffin reaction. Further, the presence of argentaffin cells does not automatically establish the diagnosis of carcinoid. A class is suggested which might be designated as "carcinoma and adenocarcinoma with included argentaffin cells." Ordinary carcinomas, adenocarcinomas and papillary carcinomas may contain considerable numbers of normal-appearing argentaffin cells interspersed among glandular and epithelial elements. The cases of Cordier [11] (adenopapilloma of the jejunum), Hamperl [12] (adenocarcinomas of the cecum, rectum and stomach, 3 cases) and Masson and Martin [13] (mucous adenocarcinoma of the pylorus with bilateral ovarian metastases) typify this variety, which would undoubtedly appear much more often if a search for argentaffin cells were made regularly.

It is probable that Pettinari's gastric "carcinoid" [14] was of this type. The Masson reaction demonstrated scattered silver reactive cells, often in clumps of 3 or 4, among the predominantly nonreactive tumor cells. It would seem that the method employed was a true argentaffin technique, since the normally argyrophil, nonargentaffin basal granular cells of the overlying mucosal glands did not react. Plaut [15] also questioned the diagnosis of carcinoid in this case. This group is excluded from further consideration among the carcinoid tumors, and the cases are not included in Table I.

Since about 30 per cent of the 183 carcinoids tested (Table I) were reported as negative by one or another silver method, it becomes necessary to scrutinize these cases particularly, to see whether or not Feyrter's statement can be confirmed. Since the existence of a positive argyrophil reaction is compatible with great depletion of the phenolic

TABLE I

SUMMARY OF REPORTS ON SILVER REACTIONS OF HUMAN CARCINOID TUMORS OF THE GASTROINTESTINAL TRACT

		Stomach	Duodenum	Jejunum	Ileum	Meckel's diverticulum	Cecum, ileocecal	Appendix	Colon	Rectum	Gallbladder	Metastasis only	Not stated	Total
Unspecified silver	+	3	0	0	0	2	1	4	0	0	0	1	0	11
	−	3	1	0	0	1	0	0	0	10	0	0	0	15
	All	6	1	0	0	3	1	4	0	10	0	1	0	26
Argentaffin	+	8	1	3	10	2	3	24	2	1	0	2	28	84
	−/?	7	0	2	1	0	3	1	2	7	0	0	0	23
	−/+	1	0	0	0	0	0	0	0	2	0	0	2	5
	−/−	1	0	0	0	1	0	1	0	3	1	0	0	7
	All	17	1	5	11	3	6	26	4	13	1	2	30	119
Argyrophil	+	1	0	3	6	0	1	20	1	0	0	0	3	35
	−/?	1	0	0	1	0	0	4	0	1	0	0	0	7
	−/+	1	0	0	0	0	0	0	0	0	0	0	0	1
	All	3	0	3	7	0	1	24	1	1	0	0	3	43
Duplication, argentaffin, argyrophil	+,+	0	0	1	0	0	0	0	0	0	0	0	0	1
	−,+	1	0	2	0	0	0	0	0	0	0	0	0	3
	−,−	1	0	0	0	0	0	0	0	0	0	0	0	1
	All	2	0	3	0	0	0	0	0	0	0	0	0	5
All silver, duplications deducted	+	11	1	5	16	4	5	48	3	1	0	3	31	128
	−	13	1	0	2	2	3	6	2	23	1	0	2	55
	All	24	2	5	18	6	8	54	5	24	1	3	33	183
No silver reaction reported	Pre-Masson	0	1	3	10	0	0	23	3	1	0	1	1	43
	Post-Masson	31*	16	27†	122*†	6	12	403	11	33	1	9	27	696
	Total	31*	17	30†	132*†	6	12	426	14	34	1	10	28	739
All cases		55*	19	35†	150*†	12	20	480	19	58	2	13	61	922

*One case, tumors of stomach and ileum. †One case, tumors of jejunum and ileum.

Twenty reports [80-99] are not elsewhere referred to in the text, since they only enumerate 116 cases in which no histochemical study was reported.

enterochromaffin substance,[16,17] positive reactions by argyrophil methods cannot be accepted as definite evidence of the presence of that substance in the tumor.

At this point some definitions are necessary. The term "argentaffin" is used to designate reactions and reactive substances in which silver salts are reduced by the tissue component to the black, presumably metallic state, without the action of any "developer" or reducing reagent applied after the exposure to silver solutions and before removal of excess silver with thiosulfate. The term "argyrophil" is used to designate those reactions and reactive substances in which a reducing agent is applied after the silver bath to build up reduced silver on nuclei formed in the tissue during the impregnation stage. Naturally all cells which have reduced silver during the impregnation stage by the argentaffin reaction remain black and are included in the total demonstrated by the argyrophil reaction, as indicated by the comparison studies of Hellweg[18] and Hamperl.[19] As pointed out on an empirical basis by Hamperl,[20] all block methods are to be regarded as argyrophil since, as we[21] have demonstrated *in vitro,* ethyl alcohol itself reduces ammoniacal silver solutions in some hours. Hence not only the Gros-Schultze and Bodian slide procedures and the Masson pyrogallol formaldehyde, the Bielschowsky and Ramón y Cajal procedures and similar postreduction block methods, but also the simple Masson[22] and Hasegawa[23] block methods, where only the dehydrating alcohol acts as a reducer, are to be classed as argyrophil methods. The distinction between argyrophil methods and argentaffin reactions lies in the fact that the argentaffin reaction, like the azo reaction, is reduced or disappears in reserpine depletion of enterochromaffin cells and in postmortem autolysis in about 6 hours. The argyrophil reaction persists apparently unimpaired in reserpine depletion and as long as cell structure remains despite postmortem change. Hence, a positive argyrophil reaction cannot be taken as evidence of the presence of any considerable quantity of the reducing substance or substances contained in enterochromaffin cells and in carcinoid tumors.

Review of Methods Employed in Cases Reported in the Literature

Argyrophil Methods

The Hasegawa[23] method was used by Erös,[24] Forbus,[25] Hasegawa,[23] McGlannan and McCleary,[26] and Schütz.[27] Raiford[28] and Gáspár[29] used both the Hasegawa and the Masson slide method.[8] Windholz[30] compared the Hasegawa and the Masson-Hamperl procedures. Methods designated as Bielschowsky, Agduhr, Gros, Foot and Ramón y Cajal were used by Wirts and Breckenridge[31] Danisch,[32] and Schack.[33] Sprafke[34] prescribed a Masson block method which included pyrogallol formaldehyde reduction, and Siburg[35] stated that he

used the same method as Sprafke. From context it is evident that the Masson method used by Humphreys [36] must have been a block method, and from its success on material taken 3 days post mortem, postreduction must be inferred. Similarly, Porter and Whelan [37] succeeded with Masson block preparations on material stored in formalin for 9 years. Normal guinea pig enterochromaffin fails to give Masson-Hamperl, ferric ferricyanide, and azo coupling reactions after 12 to 18 months in formalin. Finally, Gallinaro [38] compared the Bodian (argyrophil) and the Masson-Hamperl methods. In Schütz's case 5,[27] the Hasegawa technique was done on sections and quite possibly may have been a true argentaffin procedure. The other 4 cases had block impregnations and are to be classed as argyrophil reactions.

The argyrophil techniques have yielded 35 positive and 8 (18.6 per cent) negative reactions.

Argentaffin Methods

In 119 cases the reaction used was designated as Masson-Hamperl,[9,12,29,32–42] as Masson-Fontana or simply Fontana,[43–45] and as Masson.[3–5,46–60] In the method designated as "Bielschowsky" in the report of Hand, McCormick and Lumb,[61] Lumb writes me that the formaldehyde reduction step was omitted. Hence this was a true argentaffin procedure.

The Masson-Hamperl is clearly a true argentaffin method, as defined by Hamperl [13,30] and Cordier and Lison.[62] The Masson-Fontana and Fontana methods seem acceptable on the same basis, since the Fontana technique for spirochetes includes no postreduction step, and the original Masson slide method [8] was a true argentaffin procedure. Masson's block method with pyrogallol formaldehyde reduction is clearly an argyrophil procedure, and its nonspecificity was recognized by Masson.[22] The block method, in which only the dehydrating agents can serve as reducers, is also to be regarded as probably an argyrophil procedure since we [66] have recorded ethanol as reducing diammine silver slowly.

Since Masson [22] comments on the nonspecificity of the block procedures, it should be presumed that the specific slide method is referred to in classifying his cases as positive and negative.[3] In Bailey's series [46] the Masson slide method is clearly specified in case 3, but Bailey accepted the block and slide methods as equivalent. He notes no negative results. It appears improbable that all of the 8 carcinoids, which were incidental necropsy findings, should have been still argentaffin by the critical slide technique. Therefore, it appears that the relatively unspecific (argyrophil) block method was used when the amount of material was sufficient, and that some cases may be included as positive which might have been nonargentaffin. Horn's [50] Masson technique gave negative reactions on some of the necropsy material. No disagreement with the Gomori methenamine silver is noted. It is presumed to be a valid argentaffin method.

Acceptable argentaffin techniques have given 84 positive and 35 (32 per cent) negative reactions (Table I).

Ill-defined Silver Methods

Brunschwig and Childs,[64] Ehrlich and Hunter,[65] Paltauf [66] and Walz [67] simply recorded tumors as argentaffin or not; Marshak and Friedman,[68] Lemmer,[69] and Lützow-Holm [70] reported blackening with silver; and Entwisle [71] and Wyatt [72] recorded reduction of ammoniacal silver, without further particulars. This group includes 11 positive and 15 (58 per cent) negative reactions (Table I).

In discussing the validity of the reported reactions, it seems indicated to separate the cases into gastric; rectal; small intestine, appendix and colonic groups.

Gastric Carcinoids

Table I includes 24 cases in which silver reactions were done; these were negative in 13 and positive in 11 cases. Argentaffin cells are rare in the normal human stomach,[19,73] appearing in considerable numbers only when heterotopic intestinal glands are present, as in gastric cancer and some cases of ulcer and chronic gastritis. Hence the criterion of success on the normal argentaffin cell in the same preparation cannot be demanded for the validation of a negative Masson-Hamperl reaction of a carcinoid.

It has been widely recognized that the argentaffin substance disappears from the basal granular cells in about 6 hours post mortem.[6,19,29,84,74,75] Of Feyrter's 5 carcinoids found at necropsy, 2 were Masson-Hamperl positive, 3 were negative. The positive reactions are acceptable; the negative can be explained on the basis of autolysis (5, 10, and 17 hours post mortem).

The "Fontana"-positive tumor, with a 5-hydroxyindoleacetic acid increase in the urine, a clinical carcinoid syndrome and liver metastasis, reported by Fein and Knudtson[44] is definitely acceptable as a true argentaffin tumor. Three of Lattes and Grossi's surgical cases[43] gave weak or dubious "Fontana" reactions; one was negative. Here technical failure remains a possibility, since there is no mention of extrinsic or intrinsic (gastric enterochromaffin cells) positive control reactions. On the other hand, Gallinaro's surgical case,[66] in which both the Masson-Hamperl and the Bodian techniques demonstrated no silver-positive material in the tumor, although argyrophil (nonargentaffin) cells were demonstrated in the sides and bases of the mucosal glands, is clearly nonreactive. Lunzenauer[40] also used both the Masson-Hamperl and the Bodian procedures with negative results. In this surgical case the intrinsic control evidence of positive argyrophil cells in mucosal glands is not mentioned, and technical failure remains a possible, though perhaps improbable, explanation of the negative reactions.

Paltauf's nonargentaffin carcinoid[66] is clearly unacceptable as a proved silver-negative carcinoid, since the only material studied was serial sections of the alcohol-fixed tumor. Alcohol does not conserve enterochromaffin substance even when it contains formaldehyde. In the carcinoid syndrome case reported by Snow, Lennard-Jones, Curzon and Stacey,[45] the negative Masson-Hamperl on surgically obtained material agreed with a negative azo reaction with fast red B salt and a low assay value for 5-hydroxytryptamine in the tumor. Their other case gave positive silver and azo reactions and a 500 γ per gm. assay figure. While full details as to the technique of the positive silver reaction in Lemmer's case[60] are not available, a section method was used, and his only reference involving procedures is to Gossett and Masson.[5] Hence the tumor is to be regarded as definitely argentaffin.

Lützow-Holm's cases[70] were both surgical; both are tabulated as nonargentaffin by an unspecified technique. The silver stained granules within nuclei are not to be regarded as a positive argentaffin reaction. Moreover, neither the description nor the published photomicrographs are entirely convincing as to the identity of the 2 tumors. Despite Lattes and Grossi's[43] acceptance of these cases, they remain dubious as nonargentaffin carcinoids. In Scherman, Hara and Trafton's surgical case,[76] negative Masson ponceau acid fuchsin (trichrome?) and argentaffin stains were recorded. The method is classed as argentaffin, but no intrinsic or extrinsic controls are recorded, and technical failure remains as a possible explanation of the negative silver reaction. Walley's specimen[80] was also obtained surgically and was Masson-negative. The carcinoid structure seems acceptable. While it is not clear that Walley is speaking of his own case when he mentions the frequency of argentaffin cells in "regenerated intestinal glands" after chronic gastritis, the mention of this not-too-well known finding suggests that he saw it, and that the case must therefore be considered acceptable as a nonargentaffin carcinoid.

Wirts and Breckenridge's surgical case[87] was apparently more malignant and locally anaplastic than most carcinoids. The use of Foot's ammoniacal silver to demonstrate "many brown argentaffin granules" in the tumor is confusing, since similar silver oxide reticulum techniques often do not demonstrate normal enterochromaffin cells. If the Foot silver oxide solution was used as a true argentaffin reagent, with long exposure and no postreduction, the procedure would then be equivalent to the Masson-Hamperl method. With the formaldehyde development prescribed in the Foot and Foot-Menard techniques, the procedure is to be regarded as argyrophil. Bailey's carcinoid[46] was an apparently well preserved "incidental" necropsy specimen in which both the tumor and the enterochromaffin cells reacted to his Masson procedure. The 13 mm. diameter could have permitted use of a block impregnation. The tumor should probably be regarded as argyrophil because of the impregnation of the usually nonargentaffin, argyrophil cells of the mucosa. Heterotopic intestinal glands were not mentioned. In Pettinari's pyloric carcinoid,[14] tumor cells were positive by the Masson, Ramón y Cajal and Bielschowsky techniques, but apparently not with the del Río Hortega reticulum procedure used. The fact that no silver-positive Heidenhain cells were found in the overlying gastric mucosa indicates that a true argentaffin reaction was observed in the tumor cells. The silver-reactive cells occurred as scattered groups of 3 or 4 among the other tumor cells. The question arises as to whether the tumor belongs in the

small group cited as carcinoma with included argentaffin cells. Unfortunately, only a photostat of Pettinari's paper was available to us, and while figure 7 was suggestive of carcinoid architecture, no definite identification could be made from the illustrations. The tumor was fat-free but produced no mucus. Nevertheless, we must leave the case classed as a truly argentaffin carcinoid.

Plaut [15] questioned the diagnosis in Entwisle's surgically removed carcinoids,[71] but both the rather scanty description and the photomicrographs seem consistent. Reduction of ammoniacal silver, if taken literally, means a true argentaffin reaction. In the second case of Marshak and Friedman,[68] "staining black with silver" suggests a section method, but without further data one can only surmise that there was a true argentaffin reaction.

Summing up, the 24 gastric carcinoids have given acceptable negative silver reactions in 4 or perhaps 5 cases. There were 4 cases in which negative reactions are assignable to postmorten autolysis and to inappropriate fixation, and 3 negative and 4 feebly reactive cases in which technical failure is not excludable. Two of these cases, negative by a dubious technique, are also somewhat unconvincing morphologically. Of the 9 remaining positive cases, 5 are argentaffin, 2 probably argentaffin, and 2 possibly only argyrophil. Altogether, the tumors are predominantly argentaffin, but phases occur when the reducing material is depleted or absent.

Rectal Carcinoids

Among the 24 tumors tabulated, 23 are reported as silver-negative. The only argentaffin-positive case included in Table I is the 5 mm. nodule Brunschwig removed surgically from a point on the anterior wall, 8 cm. from the anus. Tumor cells exhibited brown to black granules, enterochromaffin cells of the crypts were black with the "Masson" technique. In view of the small size of the tumor, a slide technique appears probable, and the reaction is rated as a true argentaffin one.

Stout [50] reported very weak and negative Masson section method reactions on 2 surgically removed rectal carcinoids fixed in Bouin's fluid. The enterochromaffin cells in overlying crypts were well blackened, and despite Cordier's warning against the use of Bouin's fluid with its usual content of acetic acid, these 2 cases would appear to be valid nonargentaffin carcinoids. His cases 3 and 5, fixed with Zenker's acetic acid-bichromate sublimate fluid, were also completely silver-negative. Some brown enterochromaffin cells were recorded in case 3, but in view of the usual failure of Zenker's fluid to conserve enterochromaffin at all, the negative results on the tumor are inconclusive. Case 5, in addition, showed postmortem autolytic changes. Case 4 was a formalin-fixed surgical specimen, and the argentaffin reaction failed both in the tumor and in the enterochromaffin cells. This would appear to have been a technical failure, and the case cannot be unequivocally accepted as a nonargentaffin carcinoid. It is to be noted that Hamperl [19] also accepts only two of Stout's cases as truly nonargentaffin carcinoids.

The report of Ehrlich and Hunter [60] was a survey of 813 gastrointestinal tumors including 10 rectal carcinoids which were fat-free and nonargentaffin. The methods used are not given. The histologic appearance resembled that in Stout's cases. It is not known whether the material was procured surgically or at necropsy. Horn's case 8 [60] was a malignant carcinoid of the rectum which metastasized locally and to the liver. Tissue was obtained at necropsy. It is not stated whether the remaining 5 rectal tumors were surgical or necropsy specimens. All of the rectal tumors were negative by a Masson technique; 6 of 12 carcinoids from other areas were argentaffin. In some cases Gomori's methenamine silver [77] was used also. It would appear improbable that technical failure should select all of the rectal tumors, leaving others examined in the same laboratory reactive. Hence, unless all of the material was from necropsy specimens which had lost their reactivity by autolysis, the nonargentaffinity would seem acceptable.

The two small rectal carcinoid tumors surgically excised in the case of Martin and Dechaume,[56] and confirmed also by Masson, were presumably studied by Masson's slide method. Since Masson undoubtedly would have recognized a technical failure, this case must be accepted as a nonargentaffin carcinoid. It is to be regretted that the reactivity of the mucosal enterochromaffin cells was not noted. Siburg's case [65] of malignant carcinoid of the rectum, with local lymphatic, hepatic and vertebral metastases and verrucous mitral and aortic endocarditis, died in the evening, and necropsy was performed the next day. But the Masson technique used was the same as that used by Sprafke,[54] which included pyrogallol-formaldehyde reduction, and, as an argyrophil method, should have demon-

strated enterochromaffin cells. While these cells are not noted in the report, the reaction was regarded as successful (*"gelungen"*); hence, this case must be admitted as a non-argyrophil carcinoid.

To sum up the rectal tumors, there are on record only one argentaffin carcinoid [47] which appears quite valid, 4 clearly silver-negative ones,[25,56,59] and 4 where negative reactions are readily explained.[50,59] In the other 15 cases,[60,65] published data are not adequate to fully evaluate the reported negative reactions.

Carcinoids of the Small Intestine, Colon and Appendix

The 135 tumors tabulated include 116 silver-positive and 19 (14 per cent) silver-negative examples. In the 39 where argyrophil (usually block) techniques were used, there are 5 negatives (13 per cent). This accords rather poorly with Sprafke's statement [84] that the Masson pyrogallol-formaldehyde block method failed to demonstrate enterochromaffin cells in about one third of the relatively normal human appendixes studied. With his experience, his 2 nonargyrophil appendiceal carcinoids are scarcely significant. Siburg [35] used the same procedure on his carcinoid of the ileum, with negative results. Moreover, in this case there was postmortem autolysis, and enterochromaffin cells were not mentioned as identifiable. The Hasegawa [38] block method is regarded as an argyrophil procedure by Hamperl.[20] His only failure was on 2 malignant carcinoids of the appendix. In 5 other appendiceal and 2 ileal tumors, the reaction was positive. Hasegawa does not note whether enterochromaffin cells reacted in the 2 negative carcinoid cases. Hence technical failure is not excludable, though anaplasia remains to be considered.

In case 1 of Cruickshank and Cunningham,[48] an appendix, the Masson method failed to demonstrate argentaffin material in the Kultschitzky cells as well as in the tumor. A technical failure is indicated. Gebauer, Rümelin and Becker [39] reported a case of malignant carcinoid of the ileum with hepatic metastases, multiple small carcinoids of the jejunum, a typical carcinoid syndrome with increase of 5-hydroxyindoleacetic acid in the urine, and the pharmacologic demonstration of increased serotonin in a hepatic metastasis. Feyrter wrote me (1958) that material from this case was Masson-Hamperl negative (nonargentaffin) in his laboratory but gave a positive (argyrophil) reaction by the Gros-Schultze technique. The postmortem interval was not stated, but the photomicrographs indicate moderate autolysis. This result accords well with the dicta of Clara,[74,75] Hamperl,[20] and Feyrter [6] on the effect of postmortem autolysis on the two reactions. Windholz's case,[20] probably multiple jejunal tumors, was also negative by the Masson-Hamperl technique but positive with the Hasegawa and Bielschowsky-Maresch silver methods. Again, the stated 12 hour postmortem interval accounts for the negative argentaffin reaction and persistent argyrophilia. While Horn's reporting [50] is deficient in details regarding the possibility of autolysis (in 11 of his 18 cases we do not know whether the material was procured surgically or at necropsy), the proportion of negative silver reactions is high enough (12 in 18) to suggest some technical difficulty. However, 6 of the negative reactions are on rectal carcinoids, which have been nonargentaffin also in the hands of others. It may well be that colonic (2+:2—) and ileocecal (3+:3—) carcinoids share this tendency. Only one other colonic [85] and 2 other cecal [26,73] tumors were studied with silver; all 3 are positive. Raiford [25] used the Hasegawa (argyrophil) and Wyatt [73] an unspecified method.

Masson [8] mentioned 2 silver-negative appendiceal carcinoids among the 30 he had seen up to 1924 but gave no particulars as to site. His previous procedure had been the 1913 method [8] which was still standard in his 1923 book,[9] but at this time he had started using block impregnations and postreduction. One is inclined to accept these cases as valid nonargentaffin tumors, for surely he would have recognized technical failures. However, the influence of the postmortem autolysis had then not yet been pointed out. The negative argentaffin reaction in Ratzenhofer and Lembeck's appendiceal carcinoid [48] was clearly assignable to autolysis. The negative Masson reaction in Price's carcinoid [70] of a Meckel's diverticulum is assignable either to postmortem alterations or to the extensive necrosis which was present. Considerable cell separation is evident in the photomicrograph. Brunschwig and Childs's carcinoid of the duodenum [64] was removed surgically; it infiltrated muscle and showed a few mitotic figures. The unspecified silver reaction was negative, and the status of the Kultschitzky cells was not noted. Brunschwig [47] had previously used the Masson method and noted the reactivity of both tumor and Kultschitzky cells in a rectal carcinoid. Perhaps duodenal tumors share the proclivity of gastric carcinoids to nonargentaffinity, though this seems unlikely from my general experience with duodenal and gastric ar-

gentaffin cells in man and various mammals. However, Feyrter [6] has also reported one duodenal carcinoid in which the argentaffin reaction was positive.

Summarizing the 19 nonargentaffin carcinoids in this intestinal group, the negative reaction was assignable to autolysis in 4 cases,[20,39,45,72] possibly also in others.[8,38,80] Technical failures are indicated in 4 cases [24,25,46] and are not improbable in 9.[28,50,64] This leaves only 5 cases in which possibly valid nonargentaffin tumors have been reported.[2,28,94] One is loath to accept Masson's 2 cases [8] without more specific reporting. The possibility of valid negative reactions because of anaplasia arises in these 2 cases and in the 2 of Hasegawa [28] and that of Brunschwig and Childs.[94]

Gallbladder

Two cases of gallbladder carcinoid have been reported. Joël [51] reported the Masson reaction to be negative and noted the absence of argentaffin cells in the normal gallbladder mucosa. Some postmortem alteration is suggested by the photomicrograph, and indeed by the author, as a possible cause for the negative reaction. MacDonald [79] did not report the use of any silver reactions.

Ferric Ferricyanide Reduction

Despite Gomori's [77,100] and our [21,101,108] reports of the successful demonstration of enterochromaffin cells with the ferric ferricyanide reaction of Golodetz and Unna,[108] which is often ascribed to Schmorl, and despite Pearse's [10] commendation of the method (page 350 and Fig. 101, pages 353–354), the reaction is scarcely cited except by him as applicable to carcinoid tumors.

It is about as sensitive a reaction as either the Masson [8] or the Masson-Hamperl [30] ammoniacal silver nitrate or the Gomori-Burtner [77,100,104] hot methenamine silver methods, and does not exhibit the too frequent unexplained failures of the silver methods. Like the argentaffin reaction, and in contrast to the argyrophil method, it is rendered negative by reserpine discharge.[17] Moreover, it does not demonstrate the reportedly numerous argyrophil cells of the rat gastric fundus mucosa, and it agrees with the argentaffin and azo reactions in its susceptibility to the inclusion of alcohol in the formaldehyde fixing fluids.

Azo Coupling Reaction

The use of the azo coupling reaction of Cordier and Lison [40] has been very infrequently reported for carcinoid tumors. Pearse [10] states that staining is generally paler than in the enterochromaffin cells of the adjacent intestinal mucosa but reports no specific cases. Similarly, Gomori [100] states that it is generally weaker than in enterochromaffin cells, variable or even negative in carcinoid tumors, but gives no precise case data. Lillie's text [102] left the application of the azo reaction in carcinoids to implication. Neither Roulet [105] nor Romeis [106] report the method even for enterochromaffin.

Kahr [58] recorded positive coloration (red) with fast red B on fresh frozen tissues in one carcinoid of the ileum. Similarly, Ratzenhofer and Lembeck [42] obtained a red color with fast red B acting on slices of fresh tumor and on freshly fixed sections in their case 2 and on an extract from their case 3 (autolyzed). They noted that hot formalin fixation, or storage for 5½ months in formalin (case 1) prevented the reaction. Ritchie's review [107] mentions the reaction as positive but sometimes negative.

Gibbs's Indophenol Condensation

Gibbs's indophenol condensation with 2,6-dichloroquinonechlorimide is regarded as quite specific for enterochromaffin cells. [77] But Gomori [77] and Pearse [10] note the reaction as weaker in carcinoids than in enterochromaffin. We have found it perhaps more unimpressive on enterochromaffin than did Gomori. Although Pearse recommended it as the most specific method, it does not appear to have been used elsewhere for the diagnosis of carcinoid.

Dilute Hematoxylin

In 1935 Clara [109] reported that enterochromaffin cells were stained electively by prolonged exposures to highly dilute solutions of certain dyestuffs, all of which show *o*-diphenol

groups in their structural formulas. The dye most used was hematoxylin; gallocyanin, celestin blue, gallamine blue, alizarin cyanine RR were also effective. Gomori [77] repeated this work and added brazilin to the list of reactive dyes. We [109] have applied essentially similar methods to keratin, keratohyalin and trichohyalin. Besides these structures, which offer no confusion in the study of gastrointestinal material, eosinophil leukocyte granules react strongly, but these cells are readily distinguished by their bilobate nuclei and interstitial position. While elastic tissue reacts strongly in rodents, in man it does not. So far as we know, this reaction has not previously been applied to carcinoid tumors.

Fat in Carcinoid Tumor Cells

This was first described, according to Feyrter,[6] by Notthaft [110] who reported seeing fat granules in smears of the tumor, but did not state whether osmium tetroxide stain was used. Alkanin was known at the time, but the Sudans had not been introduced. Albrecht [111] and Maresch [112] spoke of birefringent lipoid in discussing Mandl's paper in 1911, and Oberndorfer [7] in 1912 also mentioned lipoid in discussing Saltykow's paper. A demonstration by him in 1912 is reported by Hörmann.[113]

About his own material, Feyrter [6] notes specifically one duodenal carcinoid which was heavily fat-laden, and stated that carcinoids of the jejunum and ileum contained sudanophil lipoid in about one third of the cases and birefringent crystals only in 20 per cent. Appendiceal carcinoids were often fatty; among rectal tumors, some were fatty, some were not. Lipofuscin-like pigment was occasionally present, as noted also by Masson [8] generally and by Aschoff in 1 case (cited by Feyrter [6]) and Forbus [88] in 2.

The great majority of later writers do not mention the fat content in their case descriptions, though some of them refer to it in their discussions. Masson [2,8,114] Kaufmann,[115] and Ritchie [107] speak in general terms of the frequency of neutral fat and crystalline birefringent lipoid in carcinoids. Only in 70 cases, reported by 21 authors, are specific statements made on the fat content of the individual tumors.[6,14,29,30,37,80,89,96,98,40,42,43,47,51,58,65,116–119] Birefringent lipoid, specified as crystalline or as needle-shaped crystals, was noted generally by some authors,[2,6,8,107,114,115,119] and by others [6,29,30,32,117,119] in specific cases as present in some of the fat-laden lesions and absent in others. Masson [98] characterizes the birefringent material as cholesterol esters, but does not state what tests were used.

Nile blue was used by Hada [117] and Maresch,[119] who reported pink staining, and Hada also reported a negative Fischler fatty acid test. Schütz [27] demonstrated sudanophil lipid in 4 cases, with birefringent material in 3 cases, and focally, a positive Smith-Dietrich reaction in the same 3. Nile blue staining was recorded as dirty violet in 1 case, blue in 2. Lattes and Grossi's case 5 [42] was moderately fatty, while Gallinaro [96] found little fat in a gastric carcinoid. Lunzenauer [40] and Pettinari [14] found none in their cases, and one of Feyrter's [6] was also fat-free; Siburg's case [85] and Ehrlich and Hunter's 10 cases [65] were also fat-free.

Before leaving the subject of fat in carcinoids, it is worth noting that it has usually been single cases on which the more extended tests have been done. Series have presented difficulties in that stored material is difficult to obtain or maintain and may become unreliable.

Basal Oxyphil Granulation

This conspicuous character is mentioned specifically by few authors. Often it has been quite evidently absent. Masson [8] noted it with trichrome stains, Feyrter [6] with hematoxylin and eosin stains, and Kevorkian [54] used acidulated Wright and Giemsa stains. The affinity for Altmann's aniline acid fuchsin noted by Kultschitzky [120] and other older writers for the enterochromaffin cells has been described also for carcinoids.[8,32] Retention of Heidenhain's iron hematoxylin is also noted by Masson [8] and Danisch,[32] but neither of these last techniques entails the chromation prescribed for demonstration of mitochondria by these 2 stains, and a relationship of the granules to mitochondria is not to be inferred from them. In fact Romeis [106] notes stronger eosinophilia for the enterochromaffin cells in unchromated material. The rather sketchy reports from some 25 other authors indicate pale basophilic, amphoteric or faint eosinophilic staining in about half of the cases and moderate to strong, more or less granular basal oxyphilia to eosin or the red component of the Mallory-Masson aniline blue stains.[28,29,29,31,32,39,39,42–47,50,54,59,64,70,73,107,121–129]

Heidenhain's Iron Hematoxylin

Masson [8] noted this method to be positive often and speculated on its possible relation to the argentaffin reaction. Danisch [88] reported positive results, as did others,[14,88] and von Rehren [180] accepted it as fully equivalent and substituted it for the silver reaction. This last position must be regarded as unproved and decidedly risky.

Ultraviolet Fluorescence

Ultraviolet fluorescence has been reported in carcinoid tumors, first in Erös' original report,[24] then in Hamperl's study [121] of fluorescence microscopy and later by Jacobson,[129] who noted the ultraviolet absorption maximum at 270 mμ and the maximum emission between 555 and 610 mμ. It is mentioned in Ritchie's review.[107] It seems probable that this method has not been used on carcinoid tumors more often simply because the necessary equipment was not available in operating condition at the moment.

Ninhydrin Reaction

Holcenberg and Benditt [128] reported on a new ninhydrin reaction for the enterochromaffin cells; this is said to be specific for indolethylamines. In the discussion, Dr. Holcenberg stated that carcinoid tumors gave this reaction.

Material, Methods and Results

There were in the files of the Laboratory of Pathology and Histochemistry 21 cases either diagnosed directly as carcinoid tumors or bearing diagnoses suggestive of carcinoid, which on reinspection were classified as carcinoid. Tissue containing tumor remained available as paraffin blocks in the 18 cases which are included in this study. In addition, 4 cases examined in cooperation with the Pathologic Anatomy Department of the Clinical Center are included, and one case studied by the junior author during a stay at Johns Hopkins Medical School in 1957–1958.

The older tissue was fixed in formalin and embedded in paraffin. It is to be noted that part of the older paraffin blocks were immersed in a flooded storeroom in 1942–1943, and that this immersion may account for the negative reactions in the 6 oldest cases. In all except the last 8 or 9 cases, examination of frozen section material was restricted to what was recorded at the time of original examination, because of the necessity to discard old formalin-fixed tissue.

Fresh paraffin sections were prepared and stained by the following methods. References are to pages in Lillie's 1954 text [102] or as otherwise specified.

1. For Ciaccio-positive lipids and lipofuscins: Sudan black B (p. 320) and sulfuric acid Nile blue,[134,135] 1/5000 in 1 per cent H_2SO_4 (v/v) 30 minutes.

2. For general structure and cytoplasmic eosinophilia: azure A eosin B at pH 4.5 (p. 118).

3. Reduction reactions of enterochromaffin: ferric ferricyanide (pp.

175–176); Masson-Hamperl diammine silver, 16, 24 and 32 hours at 25° C., using 5 per cent silver nitrate directly for the ammoniacal silver solution (p. 256); Gomori's methenamine silver, preheated, 2½, 3 and 4 hours at 60° C. (p. 165).

4. Azo coupling reactions with stabilized diazonium salts; fast red GG, fast red B or fast garnet GBC,[136] yielding red enterochromaffin on a light yellow background, and fast black K, yielding black enterochromaffin on a red background. One of the reds and the fast black K were used in all cases. Ice cold Michaelis veronal HCl buffer of pH 8.5 (p. 454) was used. Coupling times and concentrations were as follows: fast black K, 1 mM, 2 minutes; fast garnet GBC, 2 mM, 5 minutes; fast red B and fast red GG, 5 mM, 5 minutes. Fast dark blue R was used at 1 mM for 2 minutes in a number of cases with satisfactory immediate results, but the blue-black color faded rather rapidly in mounted preparations. Molecular weights are: fast garnet GBC, 225; fast black K, 302; fast red B, 168; fast red GG, 138. Amounts were calculated on the basis of the stated content of primary amine in the sample of diazonium salt used.

5. Gibbs's reaction with 2,6 dichloroquinonechloroimine was done according to Pearse[10] (p. 478) on 11 cases.

6. Clara's[108] dilute hematoxylin reaction was done in 18 cases. Paraffin sections were deparaffinized, hydrated, rinsed in double-distilled water and transferred to a 0.01 per cent solution of hematoxylin in double-distilled water containing M/100 phosphate buffer of pH 7. Staining was carried out for 32 hours in a covered Coplin jar in the dark. Enterochromaffin stained dark blue to black; eosinophil leukocyte granules, dark blue; nuclei were unstained to pale blue-gray; human elastic tissue was unstained; rodent elastica was blue.

Results of the foregoing studies are tabulated in Table II.

The indole reactions by the xanthydrol[137] and the "postcoupled *p*-dimethylaminobenzylidene[138] methods were done on 14 cases.

These indole reactions, which should be positive on 5-hydroxytryptamine, were almost regularly completely negative in the old material cited in Table II. The reactions with *p*-dimethylaminobenzaldehyde (Ehrlich's reagent) and with xanthydrol were also consistently negative on enterochromaffin cells.[137–143] The concentrations of 5-hydroxytryptamine in some tumors, 1 to 2.5 mg. per gm.,[42,45] should be high enough to yield directly positive reactions using ground up fresh tissue directly on a spot plate, since 0.1 cc. should contain 100 γ, and both the *p*-dimethylaminobenzaldehyde and the xanthydrol reactions are sensitive to 0.5 to 0.1 γ in spot plate tests.[137,138] Even whole proteins, such as pepsin, trypsin, lysozyme, etc., react in 10 to 100 γ quantities.[137,138]

In one of our cases (case 21) a rather weak, diffuse gray-blue staining of tumor cytoplasm was seen with the postcoupled benzylidene reaction.[188] This material was taken over 8 hours post mortem, and azo, hematoxylin, and reduction reactions revealed no granules in tumor cells. In case 20 the description of the liver metastasis fixed 3 hours post mortem for a period of 2½ hours only reads: "Liver cells are colored light gray blue, tumor cytoplasm is a faint yellowish gray with gray blue material at the border of the cells and especially basal to the nuclei of cells abutting on the tumor stroma." Duodenal enterochromaffin cells did not react, although they gave positive ferric ferricyanide, silver, azo coupling and dilute hematoxylin reactions (Table II).

Here the postmortem interval was short and formaldehyde fixation was restricted to 150 minutes. The postcoupled benzylidene reaction was much weaker in the blocks fixed for 48 hours.

The periodic acid-Schiff reaction was employed to exclude the presence of mucus in the tumor cells. Van Gieson's stain was used for demonstration of the mixed collagenous and smooth muscle character of the stroma in primary tumors.

The tyrosine method[144,145] revealed no significant concentrations of tyrosine in the 13 specimens to which it was applied.

Iron reactions with acid ferricyanide for Fe^{++} (10 cases) and ferrocyanide for Fe^{+++} (13 cases) were negative except for the interstitial pigment in appendiceal mucosa in a case of concurrent pseudomelanosis.

The eosinophilia of the basal granules at pH 4.5 in azure eosin was converted to basophilia by a 2-hour exposure to N/N $NaNO_2$/acetic at 3° C., and shifted to basophilia between pH 5.5 and 6.5. This would indicate that the basic protein probably owed its basicity largely to lysine and hydroxylysine residues.[146]

Discussion

It appears that the several reduction reactions and the azo and hematoxylin reactions are positive to about the same degree in each of the cases to which they were applied. Since there are now a few reports[42,45] and our own cases 20 and 22 in which low and high levels of 5-hydroxytryptamine assays of tumor tissue have been correlated with absence and presence of azo and reduction reactions, it would appear that the routine application of these reactions to carcinoid tumors might give some indication as to the endocrinologic activity of the neoplasm.

Since the 3 reduction reactions used appear to have essentially similar significance and sensitivity, it would not seem necessary to use all of them on each case. The ferric ferricyanide reduction test appears to be the most reliable of the 3 used, and certainly consumed the least

TABLE II

HISTOCHEMICAL REACTIONS OF 24 CARCINOID TUMORS

Case no.	Spec. no.*	Age, sex, race	Site	Invasion M S L	Median HIAA mg./day	Surg. or hr. p-m	Frozen section: Pol. Su	Pol. lt.	NB	Os	Paraf. section: Su	NB	Azure eosin: Basal gr.	Cy.	Vac.	Mit.	FF Tu	FF Ec	Diamm. silver (MH) Tu	Diamm. silver (MH) Ec	Meth. silver (GB) Tu	Meth. silver (GB) Ec	Alkal. azo coupl. Tu	Alkal. azo coupl. Ec	Hematox. (Clara) Tu	Hematox. (Clara) Ec	Indoph. cond. (Gibbs) Tu	Indoph. cond. (Gibbs) Ec
1	29428	33 MW	ap ac	− − −		S				1																		
2	30224	31 MW	ap ob	+ − −		S	1		GB		−	−	−	R∓	±	−	−		−		−		−		−			
3	31938	43 MW	ap ob	− − −		S					1	−	−	R∓	−	−	−		−		−		∓		−			
4	32303	45 MW	ap ob	+ + −		S	1	1	−				−	R∓	2	−	+											
5	553	32 MW	ap ac	− − −		S					−	−	−	R∓	−	−	−	−	1	±	−	−	−	−				
6	1176		ap	+ + −		S					1	−	−	R±	+	−	−		±		−				−			
7	4330	27 MW	ap	+ − −		S	1	1			−	−	−	R±	2	−	1	1	2	3	3	3	1	1	3	3		
8	5104	38 MW	ap	+ − −		S	1	±			−	−	−	B±	1	−	2		2		2		1		1			
9	5605	20 FI	ap ob	+ + −		S	2	2			−	−	−	B±	1	−	1	1	2	2	3	3	±	±	1	1		
10	7741	28 FI	ap	+ + −		S	±	∓			−	−	R±	B±	2	−	3	2	2	2	3	2	1	1	3	1		
11	8595	23 MW	ap ac	− − −		S					−	−	−	B+	2	−	3	3	2	3	2	3	1	1	1	1		
12	10304	41 FI	ap sa	+ − −		S					∓	±B	R	PR∓	1	−	2	2	2	3			2	1	2	±	+	−
13	13050	37 FI	ap ob	− − −		S	1				±	∓B	R+	B±	1	−	4	3	2	3			2	2	2	2	1	−
14	19707	19 MW	ap	± − −		S	2	−			−	−	R	PR±	2	−	2	2	2	2			2	1	2	2	−	−
15	24157	18 MW	ap	+ + −		S	2	−			∓	∓B	−	PR∓	2	−	−	−	∓	−			−	−	∓	−	∓	−
16	40410	54 MI	md	− − −		S							−	V±	−	−	3	3	1	2	3	3	2	2			1	1
17	44472	51 FI	ic	+ + +		S					1	1B	R	B±	−	−	2	3	2	3			2	2	2	2	1	−
18	47536	65 FI	ap ac	+ ± −		S	2	2	1B	2	1	1B	−	V±	1	−	3	−	2	−					±	−	±	−
19	A57238	50 FW	il(m)	gen.	400	18 hr.					±	±B	−	B∓	−	−	−	−	−	−	−	−	−	−	−	−		
20	A57211	53 MW	il(m)	gen.	640	3 hr.	2	−		−	−	−	−	P±	−	−	3	3	2	2	2	2	2	2	3	3		
21	A58-15	59 MW	?(m)	gen.	266	8 hr.	−	−	−	−	±	±B	−	V±	±	−	−		−		−		−		−			
22	571755	72 FW	ce(m)	gen.	6	S	2	−	−	1	−	−	−	B	1	1	−		−				−		−			
23	g176		?(m)	panc.		S							−	P±			3		3		3		2					
24	OS†	48 MN	il(m)	gen.	282		±	−	1B	−	−	−	R	V∓	−	−	2		2				2		2		1	

* Necropsy specimen numbers indicated by A; all others are surgical specimens.

† Case of Hand, McCormick and Lumb,[61] from which material was made available to us through the courtesy of Dr. Lumb.

Explanation of symbols and abbreviations

M = male; F = female
W = white; I = Indian; N = Negro
ap = appendix
md = Meckel's diverticulum
il = ileum
ic = ileocecal
ce = cecum
ac = acute appendicitis
sa = subacute appendicitis
ob = obliterative appendicitis
m = metastasis

Invasion:

M = muscularis; S = serosa;
L = regional lymph nodes
gen. = general metastasis
panc. = pancreatic region

HIAA = 5 hydroxyindoleacetic acid. Figures are the averages of the extremes recorded in each case.

S = Surgical specimen, presumably promptly fixed in formalin. With postmortem material, the number of hours postmortem is indicated.

Frozen sections:

Su = Sudan IV, oil red O, or Sudan black B
Pol. lt. = examination under polarized light (Amounts: ∓, trace; ±, little; 1, moderate; 2, large)
NB = Nile blue (B, blue; GB, green blue)
Os = osmic acid reduction

Paraffin sections:

Su = usually Sudan black B
NB = Nile blue sulfuric method
Basal gr. = basal granulation of tumor cells
Cy. = general tumor cell cytoplasm (R, red; R±, pink; R∓, pale pink; P, reddish purple; P±, purplish pink; V, violet; B, blue)
Vac. = cytoplasmic vacuolation
Mit. = mitotic figures
FF = ferric ferricyanide
Tu = tumor cell reaction
Ec = enterochromaffin cell reaction
(MH) Masson Hamperl, and (GB) Burtner and Lillie variants of Gomori's methenamine silver for enterochromaffin cells.
Hemat. = hematoxylin (Clara)
Indoph. cond. = Gibbs's indophenol condensation

(Intensities: −, ∓, ±, +, 1, 2, 3, 4)

working time in its execution. Similarly, the azo reaction, Clara's hematoxylin, and to a less extent the Gibbs reaction, afforded more specific though less sensitive tests for the enterochromaffin substance.

In our hands the Gibbs method has been disappointing in its intensity and discernibility. Clara's hematoxylin reaction often gave brilliant results, but its optimum time was long and somewhat uncertain, 24 to 48 hours covering the usual range. We have had relatively constant and brilliant results with the azo reaction. Of the many diazonium salts tested, those giving deep red colors on a light yellow background gave the most easily recognized positive reactions. Among these we have come to prefer fast garnet GBC at 1 mM concentration (45 mg. per 40 cc.) in pH 8.5 veronal HCl buffer, allowing optimally about 2 minutes at 3° C. for coupling. We then washed in 3 changes of 0.1 N HCl, 5 minutes each, and 10 minutes in running water. A 2 minutes' alum hematoxylin counterstain may be used; we usually omitted it.

It has proved possible to restore the reactivity to the azo and hematoxylin reactions of enterochromaffin. After even very old silver impregnations and other oxidative treatments, the masking silver deposits may be removed with potassium cyanide solution and the enterochromaffin substance reduced from its presumed quinonoid form with sodium hydrosulfite ($Na_2S_2O_4$). Since old silver-impregnated blocks, even when they have failed to demonstrate enterochromaffin satisfactorily, may be better preserved histologically than plain formalin-fixed material, these blocks may thus afford the opportunity for demonstration of the relatively specific azo reaction. This reduction procedure was carried out successfully on a number of human appendix blocks impregnated by the Masson procedure (p. 101).[147]

The technique for this procedure: Deparaffinized, hydrated sections are immersed in 5 per cent KCN for 30 minutes, washed 5 minutes in water, immersed in 2 per cent $Na_2S_2O_4$ for 6 hours, running water for 5 minutes, 45 mg. of fast garnet GBC in 40 cc. ice cold veronal HCl buffer (pH 8.5) for 2 minutes, washed in 3 changes of 0.1 N HCl for 5 minutes each and in running water 10 minutes. Alum hematoxylin was used if counterstain was desired, 2 minutes, alcohols, xylene, xylene-cellulose caprate. Background stained yellow; enterochromaffin, dark red; nuclei, blue if hematoxylin was used; otherwise they were yellow.

It is further obvious from the histochemical study of carcinoid tumors that they do not represent a single cell type from the intestinal mucosa. Fat in carcinoid tumors has been quite frequent in its occurrence, though a few fat-free tumors are reported. The fat apparently includes neutral fat, a quite variable amount of relatively sudanophobic, birefringent needle-like crystals, and an insoluble lipofuscin-like material

which may or may not attain a yellow brown color. This combination of fatty substances is reminiscent of those in adrenal cortex and corpus luteum. We are at a loss to assign an origin for fat-forming cells of such a type among the normal cell population of the gastrointestinal mucosa. The enterochromaffin cells have been repeatedly reported as fat free.[6,12,148–150] Our own experience in dogs and guinea pigs confirms this. And the enterochromaffin cells of the overlying mucosa in cases of carcinoid tumors are not demonstrated by the oil soluble dyes. Only Zanardi[151] has reported sudanophilic droplets in enterochromaffin cells in frozen sections which had been presilvered by a Masson technique with or without gold toning. Feyrter and Unna[152] made the same comment that we did ourselves in the original protocol of the first case included in this series, that it bore more resemblance to an adrenal cortical tumor than to a pheochromocytoma.

Positive Smith-Dietrich tests for phospholipids have been reported for enterochromaffin by Christie,[153] Erös,[154] Hada[117] and Pavone[148]; but in 1906, Harvey[155] wrote that the chromaffin cells of the dog stomach when stained by copper chromium hematoxylin (we infer after chromate fixation) were readily decolorized by Weigert's borax ferricyanide mixture, being decolorized apparently before the parietal cells. Our own experience has been that guinea pig duodenal enterochromaffin cells decolorized along with cell nuclei and before erythrocytes.

The relation of the Smith-Dietrich reaction to the often reported black staining of the granules by Heidenhain's iron hematoxylin, and for that matter, by Altmann's aniline acid fuchsin in his mitochondrial technique, is uncertain. In the face of the negative Sudan staining, it remains dubious that the test indicates phospholipid. Extractability with hot pyridine[153] is certainly not an exclusive property of lipoids. Many phenolic substances are soluble in nonpolar solvents. And the enterochromaffin substance is also removed or rendered nonreactive by prolonged exposure to cold formaldehyde and short exposure to hot formaldehyde solutions and to hot 60 per cent alcohol, according to Ratzenhofer and Lembeck.[42] We confirmed their experience with formaldehyde and here record further that a 10 to 30-minute exposure to distilled water at 95° C. also abolished the reactivity of formaldehyde-fixed guinea pig duodenal enterochromaffin. A 16-hour exposure at 60° C. was largely effective, and similar exposures to strong alcohol greatly reduced the number of reactive cells. The ferric ferricyanide and Masson-Hamperl reactions and azo coupling with fast red B and GG were used as the demonstration methods.

In any case the lipoids seen in carcinoid tumors were largely neutral fat and quickly soluble, birefringent, aciform crystals, which were not

demonstrable in enterochromaffin cells, if one ignores Zanardi's unconfirmed report.[151] However, the presence of some sudanophil lipoid in carcinoid tumors was often demonstrable also in paraffin sections, and this was sometimes quite definitely of the lipofuscin fatty acid variety by the sulfuric acid Nile blue method.

Pearse's demonstration[41] of peptidase (or esterase) in carcinoid tumors and its absence in enterochromaffin cells is further evidence that the carcinoid tumor does not represent a single cell type from the gastrointestinal mucosa.

Strong eosinophilia in well localized basal granules was evident in a minority of carcinoid tumors. This was readily demonstrated with buffered azure eosin stains at pH 4.5 as well as with hematoxylin and eosin. Most enterochromaffin cells were not demonstrable by this technique, although Kevorkian[54] had considerable success with Romanovsky stains for this purpose. However, Kevorkian did not adequately control his studies by using matched serial paraffin sections stained by his methods and by one of the recognized specific methods (argentaffin, ferric ferricyanide or azo). It is tempting to assign this eosinophilic granulation to a possible Paneth cell or related stem cell participation in the carcinogenetic process. The eosinophilia of carcinoids and of Paneth cell granules disappeared similarly when the stain pH was raised to about 6.0, and relatively brief (2 hour) nitrosation destroyed the eosinophilia at pH 4.5 in both instances. Of course there are a considerable number of other basic protein sites which exhibit similar behavior in normal animals.[145]

Mucous globules were usually absent from carcinoid tumors, and it is probable that their presence in any appreciable number would lead to classification of the tumor as adenocarcinoma. There have been reported 5 cases which we have excluded from the general tabulation in Table I and have referred to as "carcinoma and adenocarcinoma with included argentaffin cells" (stomach, 2; jejunum, cecum, and rectum, 1 each).[11–13] It seems highly probable that this class of tumors would increase in number if a routine search for enterochromaffin substance were made in them also, as we have suggested for the carcinoid tumors.

The demonstration of indole reactive material in formaldehyde-fixed carcinoid tumor tissue by the postcoupled benzylidene reaction agreed with the assays (80 γ to 2.5 mg. per gm.) reported for 5-hydroxytryptamine in tumor substance reported by a number of workers.[39,42,45,52,53,61,156] It is noteworthy that our best success with this reaction was on material taken only 3 hours post mortem and fixed promptly for a period of only 150 minutes in calcium acetate formalin. A similar success with canine and mouse mastocytoma has been related

recently by Meier [157] who reported blue staining of granules and lighter staining in cytoplasm, with assays ranging from only 0.29 to 0.83 γ per gm. in dogs, as high as 28 γ per gm. in the mouse.

In view of these successes and the uniform failure of the *p*-dimethylaminobenzaldehyde reaction and its variants on the enterochromaffin cells, one is again led to wonder whether or not serotonin is produced in the enterochromaffin cells, or perhaps in some other cell component of the intestinal mucosa. We [17] have commented previously on the lack of numerical correspondence of the enterochromaffin cells of the guinea pig intestine with the serotonin assays. The one conspicuous site of positive reactivity to the postcoupled benzylidene reaction in the human and guinea pig duodenum is the Paneth cell. This we have usually assigned to protein bound tryptophan in the presumed enzyme content of the Paneth cell granule. The Paneth cell, however, is generally considered an exocrine cell, both because of its structure and its cyclic depletion and refilling in relation to food intake. In the same paper we recorded that the Paneth cell granules were not regularly discharged by large doses (LD/50) of physostigmine, or to any evident degree by doses of reserpine sufficient to completely deplete the enterochromaffin cells. Thus, the idea that serotonin might be produced by Paneth cells is not supported by the pharmacologic experiments.

Conclusions

Nearly all carcinoids of the small intestine, appendix and cecum are argentaffin when properly preserved by prompt formaldehyde fixation. The same tumors quite regularly give ferric ferricyanide reduction, positive azo coupling and hematoxylin condensation reactions.

The ferric ferricyanide reaction is about as sensitive as the argentaffin reaction and appears much more reliable and easier to perform. The azo coupling reaction is probably the most specific of the commonly applied reactions and is easy and quite reliable in performance when stabilized diazonium salts are employed. The routine application of these two reactions to carcinoid and similar appearing tumors is urged, in order to accumulate further data on tumors with probable endocrine activity which give histologic evidence of production of phenolic substances. Particularly is such study urged for gastric and rectal carcinoids where the silver reactions are, respectively, often and usually negative.

Other histochemical evidence, i.e., eosinophilia, varied lipoid content and the presence of esterase, suggests a more complex origin of carcinoid tumors than from the enterochromaffin cells alone. The lipoid content would appear to merit much more detailed chemical study than it has

received. An indolic substance, not improbably 5-hydroxytryptamine, may be localized in the basal granules of some carcinoid tumors when appropriately brief formaldehyde fixation is applied to quite fresh, unautolyzed material. The identification of this substance with the definitely phenolic enterochromaffin substance remains uncertain.

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[*Illustrations follow*]

Legends for Figures

Fig. 1. Fat stain (oil red O) showing fat droplets in the cytoplasm of carcinoid cells. Counterstained with hematoxylin. × 285.

Fig. 2. Azure eosin stain, pH 4.5, showing basal staining of peripheral cells in a carcinoid tumor. × 210.

Fig. 3. A methenamine silver reduction reaction localized predominantly to cytoplasm of peripheral cells in a carcinoid tumor. × 210.

Fig. 4. The ferric ferricyanide reaction is localized predominantly to cytoplasmic granules of peripheral cells in a carcinoid tumor, but it is also present in central tumor cells. × 210.

Fig. 5. The diazonium salt coupling reaction is localized predominantly to the periphery of tumor cell clumps. × 210.

Fig. 6 Clara's hematoxylin reaction is localized predominantly to the periphery of tumor cell clumps. × 210.


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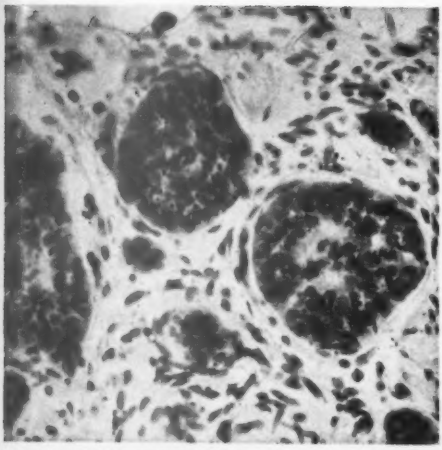

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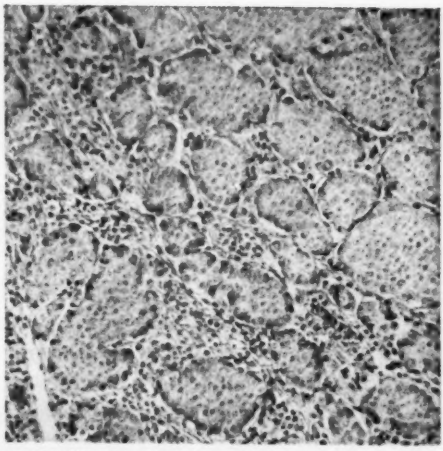

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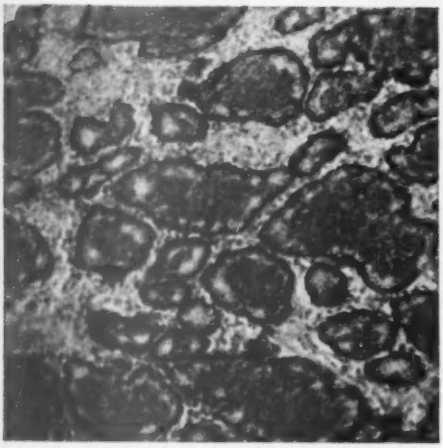

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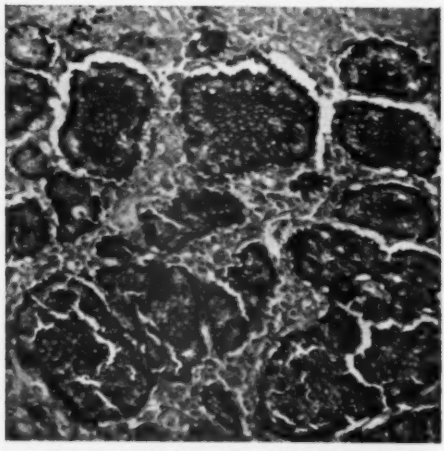

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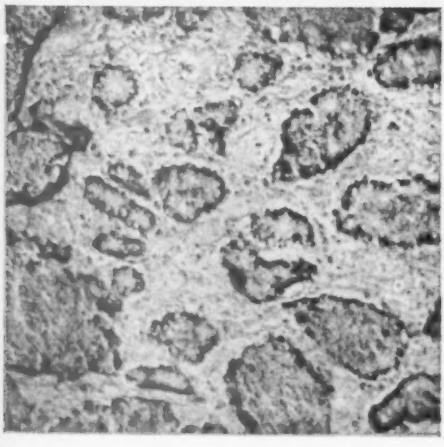

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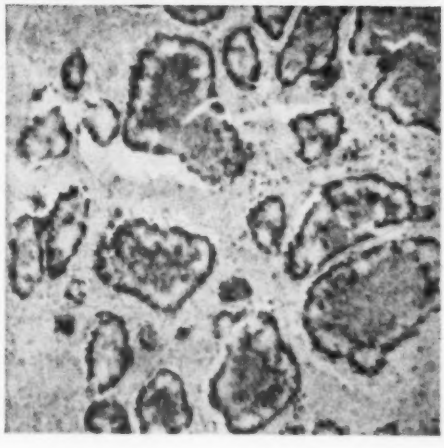

6

THE PRODUCTION OF BRONCHIAL CARCINOMAS IN MICE

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The growth of the atomic energy industry, the resultant possibility of escape of radioactive wastes into the atmosphere, and the presence in air of particulate radioactive fallout make it desirable to understand more fully the role of radiation in the production of pulmonary damage and of lung tumors. Radioactive particles suspended after an accidental atomic explosion or during nuclear tests or warfare are not an immediate inhalation hazard, but their possible late effects on the lung have not been fully evaluated. Levels of radioactivity sufficient to cause acute pulmonary damage would be reached only when there were simultaneous supralethal doses of external beta-gamma radiation.[1]

Particulate material, whether radioactive or not, that reaches the lung by inhalation is small in size (5 μ or less) and tends to be removed, at least in part, by phagocytosis, by entrapment in mucus with transport by ciliary action, and by some degree of solution and absorption. Some phagocytosed material may be held in alveolar walls, beneath the pleura or in regional lymph nodes. The high incidence of lung (bronchial) cancer in the Joachimsthal miners breathing radon-laden air has long been known. Most of the radiation hazard of inhaled radon is due to its daughter products plated out on dust particles.[2] External radiation has not produced pulmonary or bronchial tumors in man. Warren and Gates[3] called attention to the pathology of radiation pneumonitis in man and animals, and pointed out the bizarre cellular changes present, but found no induced tumors among the cases studied.

Even though ionizing radiation is a potent carcinogen in mice, and to a lesser degree in rats, we have not induced cancers of the bronchial epithelium by external radiation or found records of tumors so induced.[4] Spontaneous bronchial carcinomas resembling those in man have not been found in rodents, although alveolar adenomas and even adenocar-

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Presented in part at the Fifty-sixth Annual Meeting of the American Association of Pathologists and Bacteriologists, Boston, Mass., April 23, 1959.

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cinomas appear in some strains of mice. These have been shown to increase in frequency of occurrence following application of some chemical carcinogens elsewhere than the lung.[5,6] Epidermoid carcinoma has followed direct application of a chemical carcinogen to the bronchial epithelium of the hamster.[7]

Bronchial epidermoid carcinomas have rarely been induced by internal radiation in rats, even more rarely in mice. We have induced bronchial carcinomas in mice by implantation of radioactive cobalt, an emitter of penetrating gamma rays.

Review of the literature suggests that it may be easier to produce bronchial carcinomas in rats than in mice. Chronic murine pneumonia is common (50 to 75 per cent) in rats, and is associated with patchy pneumonitis and bronchiectasis, more rarely with squamous metaplasia of bronchial epithelium.[8] These lesions might predispose to radiation carcinogenesis. Neither our control mice nor the less heavily irradiated portions of the lungs of the experimental mice showed evidence of such infection, although abscesses or pneumonic foci were occasionally found. Most of the deaths from pulmonary infection occurred within the first month of the experiment. When carcinoma developed, it was not associated with infection.

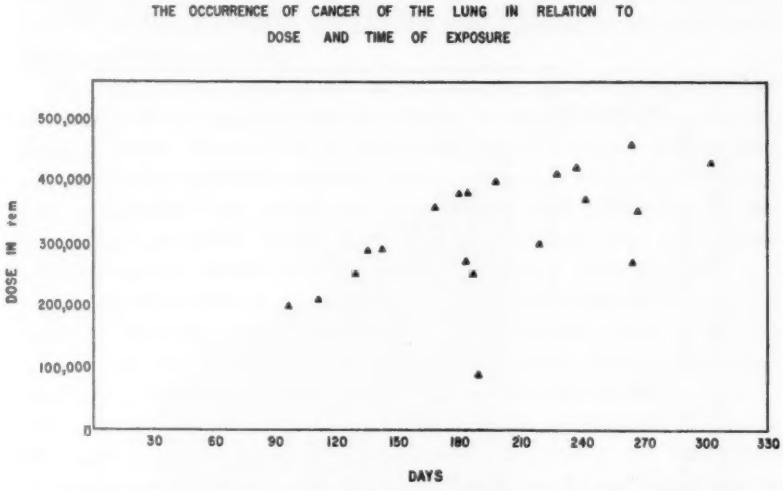
Material and Method

By means of a trocar, cobalt 60 alloy wire, about 2 mm. long by 0.5 mm. thick, or glass beads about 0.7 mm. in diameter, containing strontium 90, were placed in the right lung or pleural cavity. The cobalt wire implants had an activity of 0.25 to 0.17 mc. The size was small enough to be considered as a point source. The experimental subjects were Rockland All Purpose (RAP) mice, a variant of the Swiss strain. The mice were from 4 to 6 weeks old, and both sexes were used. In over 2,000 RAP mice, treated with iodine 131 for another experiment, tumors were rare up to one year of age; none occurred in the lung, and only one epidermoid carcinoma of the skin was found.[9] The commonest spontaneous tumor noted in RAP mice is mammary in type, and its incidence is low.[10] Thus, we feel those tumors found in our series may be considered to be radiation-induced.

The total radiation received in various tissues or organs was figured on the basis of the accumulated radiation received by the nearest portion of that tissue or organ to the source. This did not include the most proximal 0.2 mm. of the tissue, as this was usually necrotic (Fig. 1). Sometimes the radioactive source was found in muscle, fascia, bone, or subcutaneous region at necropsy, due either to initial misplacement or necrosis and subsequent migration along tissue planes. In one animal

the source had sloughed into the esophagus after 102 days and was passed in the feces.

The rate of radiation 2 mm. from the source was about 2,000 rem per day; at 5 mm. it was 250 rem; and at 10 mm., 75 rem per day. Radiation from Co^{60} is highly penetrating; the conversion factor to rads in water



TEXT-FIGURE 1. The induction time for cancer of the lung in this series is at least 90 days, and the mean time lies between 180 and 200 days.

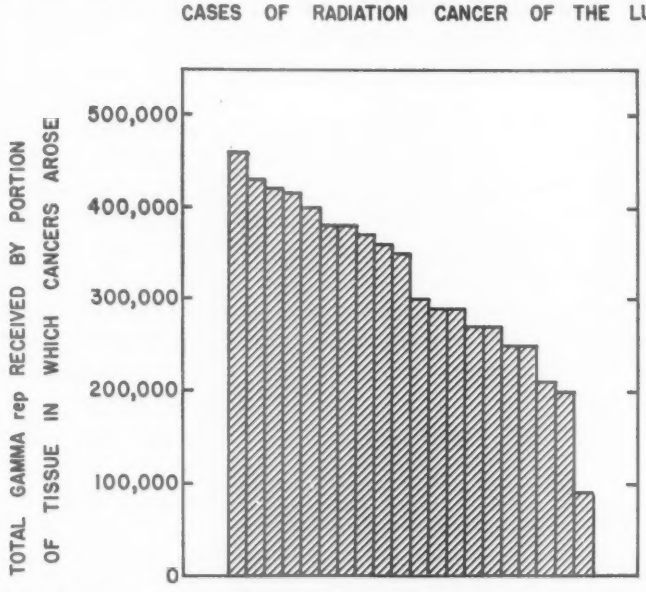
is 0.979. The relative biologic effectiveness is 0.85.[11] The beta radiation component of Co^{60} was not included in the dose calculated. Most of this does not escape from the wire because of absorption within the alloy, and that which does fails to penetrate beyond the zone of necrosis.

The glass beads contained from 0.106 to 80 μc of Sr^{90}-Yt^{90} and emitted beta radiation at rates from less than 1 rem to 540 rem per day. Practically none of this beta radiation reached tissues beyond one centimeter from the source. The use of the beads was accompanied by much more sepsis than the Co^{60} wires, although the same technique of implantation was used (Fig. 2). Only 13 animals survived beyond 11 days, and 4 beyond 100 days.

RESULTS

The present investigation is based chiefly on mice that lived over 97 days after the implantation of the cobalt source, the shortest time at which lung cancer was found being 97 days. The oldest died 315 days after implantation. Twenty of the 190 Co^{60}-treated mice living 97 days or more developed carcinoma of the lung or bronchus. Text-figure 1

shows the occurrence of cancer of the lung in relation to accumulated dose and time of exposure. The lung cancer in most animals was still early and was rarely the immediate cause of death. Hence, while there was some lag between the induction of lung tumor and death, this should not have been great in proportion to the total time and dose. However,



TEXT-FIGURE 2. High doses are required for the induction of lung tumor; only one followed a dose less than 200,000 rep.

since the radiation was continuous until the death of the animal, there may have been some "wasted" radiation.[12] This was probably not great; many animals that received 300,000 to 450,000 rem did not develop lung cancer, and most of the cancers found were small and free from necrosis, implying that they were of recent origin.

The estimated doses of radiation delivered to the most heavily exposed but viable cells of the lung in the 190 mice ranged from 80,000 to 460,000 rem, and in most animals was over 200,000 rem. The lowest dose associated with lung cancer was 90,000 rem, the highest 460,000 rem (Text-fig. 2). In the animal receiving only 90,000 rem, the dose was estimated. The cobalt wire here was assumed to have sloughed out of the lung, and was found embedded in a necrotic focus in the muscles of the neck. This dose was minimal, and if the source had not migrated at the time estimated, it would have been much higher. The lung showed radiation pneumonitis as well as cancer.

In general, alveolar and endothelial cells deviated most markedly from the normal in the irradiated lung, but we found no evidence of neoplasms arising from these cells. While the bronchial epithelium showed well defined alterations due to radiation, the cells were neither as consistently nor as markedly changed. Most animals showed highly bizarre cells and giant cells in foci of radiation pneumonitis, but these cells were neither invasive nor actively proliferating; hence, they were not considered neoplastic.

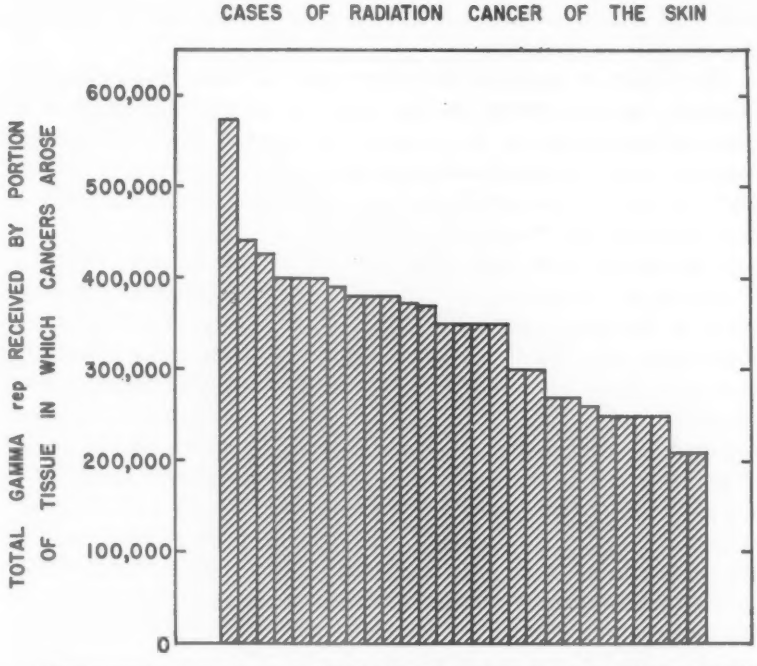
The tumors developed in the narrow zone of viable tissue which had obviously been irradiated but not wholly in regions with the most intense radiation reaction. In the latter the lung was largely fibrotic and contained only scattered persisting bizarre cells, which were for the most part endothelial. The neoplasms were epidermoid carcinomas; the degree of keratinization ranged from complete to slight (Fig. 3). Mitotic activity was not great, occurring in less than 5 per thousand cells.

Few of the tumors were recognizable grossly although they were obvious to the naked eye as foci of pathologic change. Even under the microscope, some were small (less than a low-power field). Many cancers were clearly multicentric; others were probably so. Most of them appeared to arise from small branches of the bronchial tree. Although the exact point of origin could rarely be demonstrated, the epithelium of major bronchi, terminal bronchi and atria was clearly identified as the source of the neoplasm in some instances. So-called precancerous epithelial alterations were usually associated with the cancers but were seen in more cases than were the tumors (Fig. 4). These changes were fully as conspicuous in cells lining the alveoli as in the bronchial tree. Our observations did not provide indications of the significance of the rather marked alterations of alveolar lining cells or a clue to whether they were truly epithelial or mesenchymal. Squamous metaplasia without anaplasia was seen in only a few instances and seemed to be secondary to infection. It had no apparent relation to the development of neoplasm. The cancers not infrequently extended fairly equally into the alveoli of a given branch or branches (Figs. 5 and 6). At times, the tumors clearly originated from bronchial epithelium and filled the lumen to a greater or less degree before invading the parenchyma extensively (Fig. 7). None of the neoplasms metastasized, unless some of the multiple foci in the lung, interpreted as primary lesions, could have been metastatic.

One mouse showed a pulmonary adenoma which was assumed to be spontaneous, and in two instances pulmonary metastasis from breast cancer was found. Many mice showed leukemic involvement of the lung, and tumors of other tissues were also found.

Many animals were not included in the study, as they died before 97 days from infection, leukemia, paralysis due to radiation-induced transverse myelitis, and from radiation esophagitis.

Four tissues exposed to radiation by Co^{60}, skin, lung, hematopoietic organs and esophagus, developed numbers of tumors. Epidermoid car-



TEXT-FIGURE 3. Since cancer of the skin grows very slowly in mice and since the skin is continuously irradiated by the radiation source, high dose levels are reached before the animal dies.

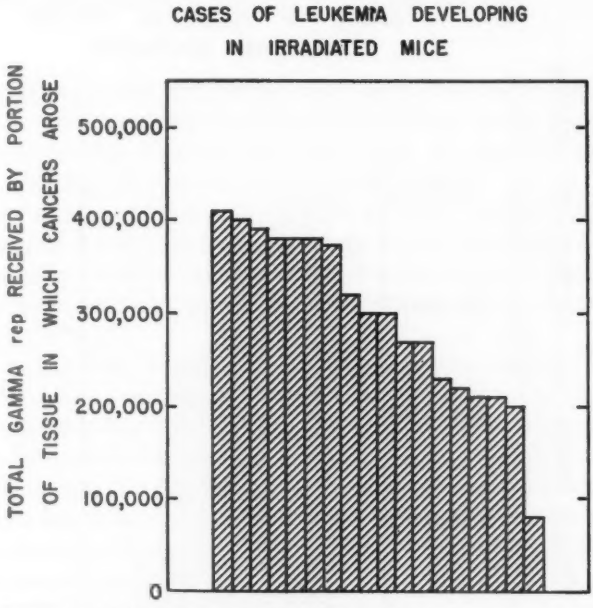
cinoma of the skin was common, appearing in 26 animals living 97 days or over. In these the skin had received total doses ranging from 210,000 to 572,000 rem (Text-fig. 3). A portion of the total radiation dose to the skin was wasted, as we found evidence that a number of skin tumors developed some time before death; some even showed evidence of focal destruction by the radiation. All doses, however, were calculated up to the time of death of the animal.

Leukemia with thymic involvement or exhibiting myeloid or reticulum cell pattern had killed only 18 mice in this group (Text-fig. 4). Only two had died of leukemia prior to the induction period of lung cancer and were not included. Esophageal carcinomas (Fig. 8; Text-fig. 5)

were least frequent (9), but severe radiation reaction and precancerous changes often led to malfunction of the esophagus. There was even occlusion by keratinized plugs and death from malnutrition.

While the accumulated doses of radiation to different tissues varied somewhat, most animals received over 200,000 rem to all. Hence, it would appear that in RAP mice, radiation induces skin cancer most readily, lung cancer and leukemia at about the same rate, and affects the esophagus least frequently.

The Sr^{90} beads did not produce lung cancer, but did cause radiation pneumonitis. Most animals did not live long enough. The heaviest radiation to the lung from Sr^{90} was 360,000 rem in an animal living 110 days. This animal had a very marked pulmonary radiation reaction, but can-



TEXT-FIGURE 4. Only those cases of leukemia developing in mice living beyond 90 days are indicated. Rare instances occurred prior to this time.

cer developed in the esophagus, which had received 300,000 rem. Slight radiation reaction in the lung appeared after 4,800 rem and 23 days, or 7,000 rem and 13 days.

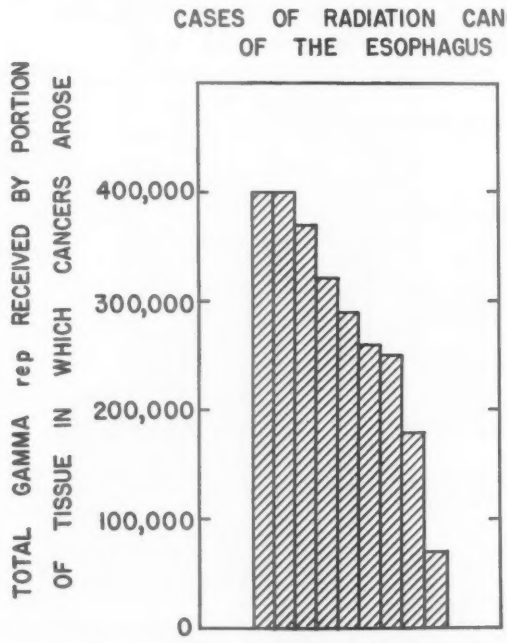
Two cases of lymphatic leukemia occurred. One was observed after 475 days with a total accumulated dose to potentially leukemogenic tissue of 1,500 rem, the other after 833 days and 4,000 rem. Epidermoid cancer of the skin was found in an animal living 475 days with a skin

dose of 18,000 rem. An esophageal epidermoid carcinoma was encountered at 110 days; the dosage was 300,000 rem.

Discussion

Several other radioactive substances have induced lung cancer. Direct action of particulate alpha emitters, such as plutonium [13,14] and polonium [15] on bronchial epithelium has produced bronchial epidermoid carcinoma in rats and mice. The induction of bronchogenic carcinomas in rats by inhalation of radioactive cerium oxide fumes emitting beta particles and gamma rays has also been reported.[16]

Cember [17] did not find either carcinoma of the lung or radiation pneumonitis in rats which had inhaled barium sulfate containing beta-



Text-figure 5. Cancer of the esophagus is induced by doses in the range of 400,000 rep or less. It is less frequent in this series than the other types of neoplasm reported.

emitting sulfur 35, giving local doses of 24,000 beta rep. However, with repeated insufflations and a total dose average to the entire lung below 20,000 rep, he obtained radiation pneumonitis and epidermoid carcinoma of the bronchus.[18] Much higher local doses must have been delivered in close proximity to active particles. Inhaled particles coated with Tl^{204} did not cause radiation damage.[19] Sr^{90}, another beta emitter,

was implanted in bead form by Cember and Watson[20] in the lungs of rats. Doses ranging from 78,000 to 260,000 rads resulted in epidermoid carcinoma of the bronchus in 4 animals.

By implanting pellets emitting beta radiation of ruthenium 106-rhodium 106 in the bronchus of rats, Kuschner and his associates[21] induced squamous metaplasia and epidermoid carcinoma at dose levels estimated at about 200,000 rads. Temple, Willard, Marks and Bair[13] recorded two lung cancers from insufflation of $Ru^{106}O_2$ particles.

Lung cancers have been produced, as stated above, by alpha, beta and gamma emitting sources of radiation. Dosimetry of various exposed organs and tissues is more readily determined with the use of gamma sources. Stannard[22] provided a useful summarization of the radiation dose-effect relationships of the lung in man and animals.

Summary

Broncho-pulmonary carcinomas have been induced by an internal emitter of gamma radiation, cobalt 60. This isotope has not previously been used for this purpose, but other types of internal radioactive sources have been successfully employed by others.

Beads containing Sr^{90} did not cause lung tumors, but did produce one carcinoma of the skin and one carcinoma of the esophagus. However, too few animals lived sufficiently long to give these findings significance. The radiation reactions found were histologically similar to those produced by Co^{60}.

Our observations indicate that continuous gamma radiation in heavy doses will cause carcinoma of bronchial and atrial cells. The pulmonary neoplasms were less common than cancer of the skin in this series of mice, even though lung tissue received heavier or as heavy doses, compared to the skin. The duration and rates of exposure were comparable. Leukemia was no more frequent than was lung cancer, and its incidence was not related to the dose in linear fashion. We believe more esophageal cancers would have developed had not some mice died as a result of severe radiation reaction of the esophagus.

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[*Illustrations follow*]

Legends for Figures

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

Fig. 1. Lateral view of the right lung, showing a Co^{60} wire partly embedded in the parenchyma. Note the shrinkage of a portion of the upper lobe and a zone of necrosis immediately about the Co^{60} wire. Total dose, 430,000 rem over 303 days. × 2.

Fig. 2. Posterior view of the lungs, showing acute infectious pneumonitis and a defect at the site of a Sr^{90} bead. Total dose to lung, 7,000 rem over 13 days. × 2.

Fig. 3. Epidermoid carcinoma of the bronchus, invading the parenchyma of the lung and showing keratinization. Total dose, 350,000 rem over 260 days. × 500.

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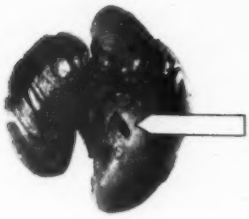

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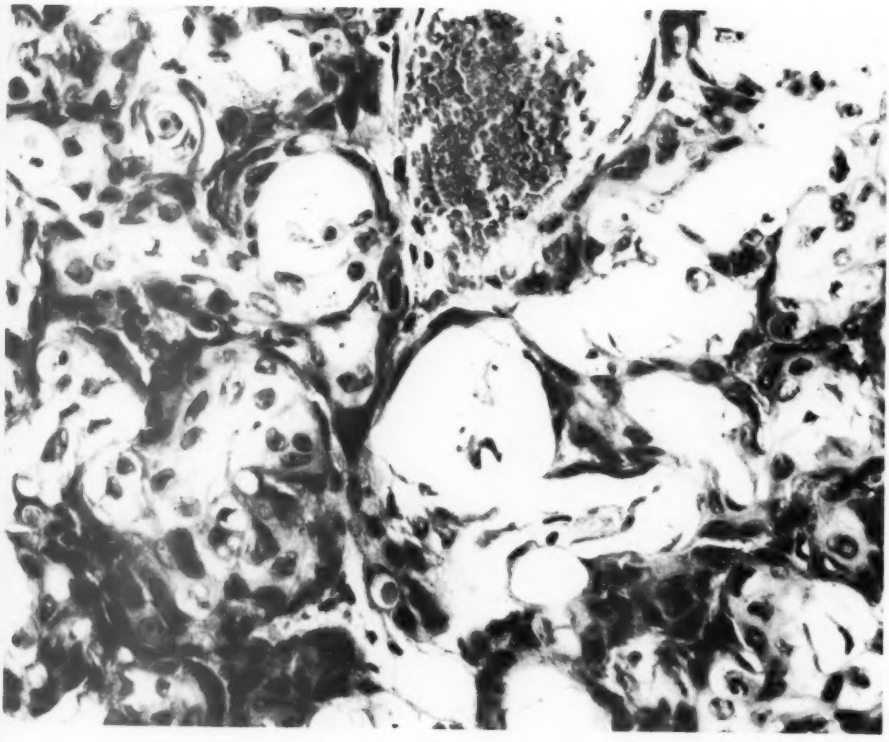

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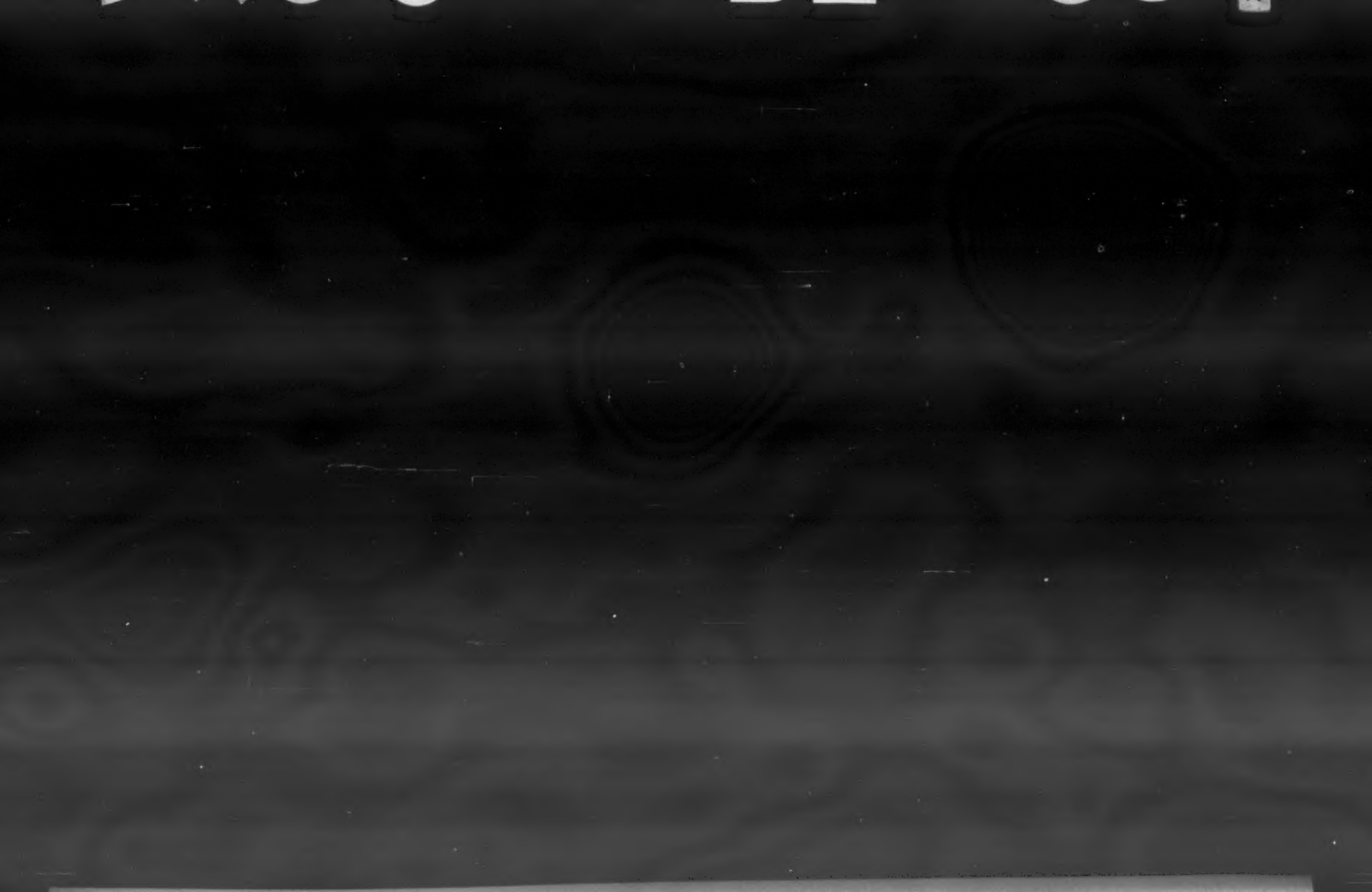
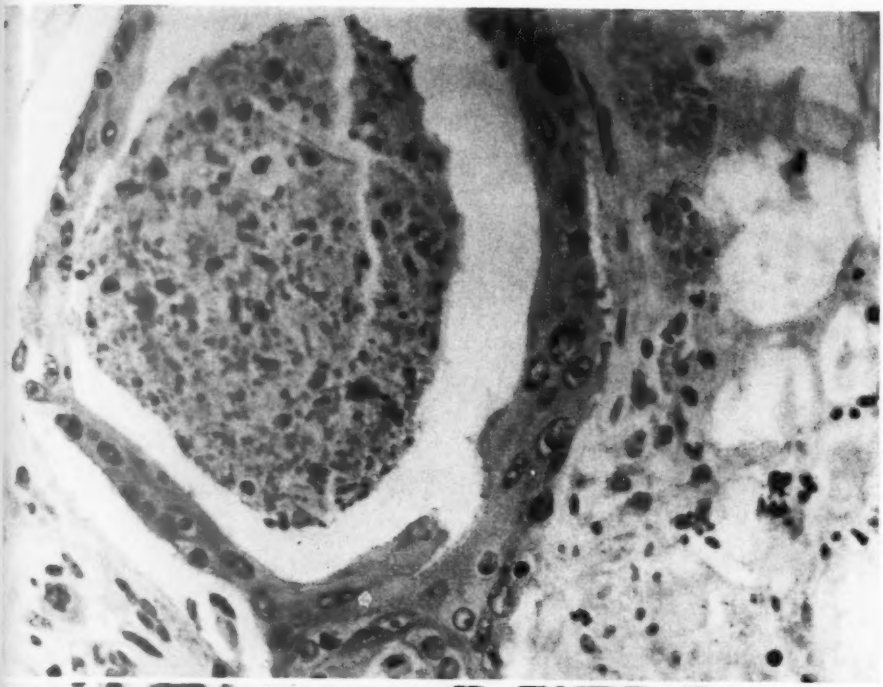
FIG. 4. A portion of a bronchus, showing precancerous epithelial changes and radiation reaction in the adjacent parenchyma. This tissue received a total dose of 400,000 rem during 199 days. Cancer was present elsewhere in the lung. × 500.

FIG. 5. Epidermoid carcinoma, showing origin from a bronchiole and extension fairly equally into adjacent alveoli. Total dose, 350,000 rem over 266 days. × 500.





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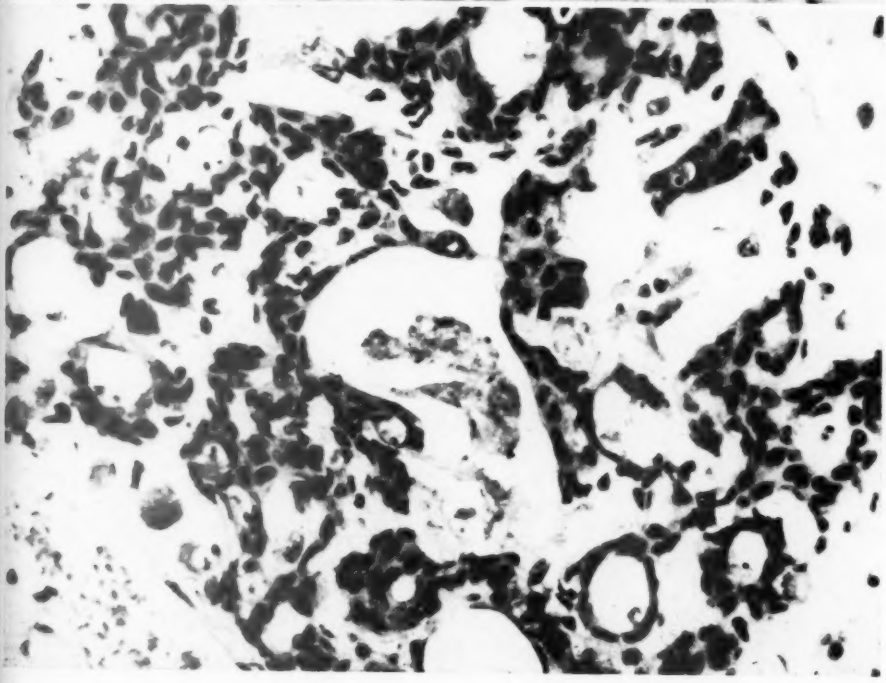

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FIG. 6. An epidermoid carcinoma with varying degrees of keratinization extending from a bronchus. Total dose, 415,000 rem over 223 days. × 250.

FIG. 7. An epidermoid carcinoma arising from bronchial epithelium and occluding the bronchial lumen. Total dose, 380,000 rem over 180 days. × 500.

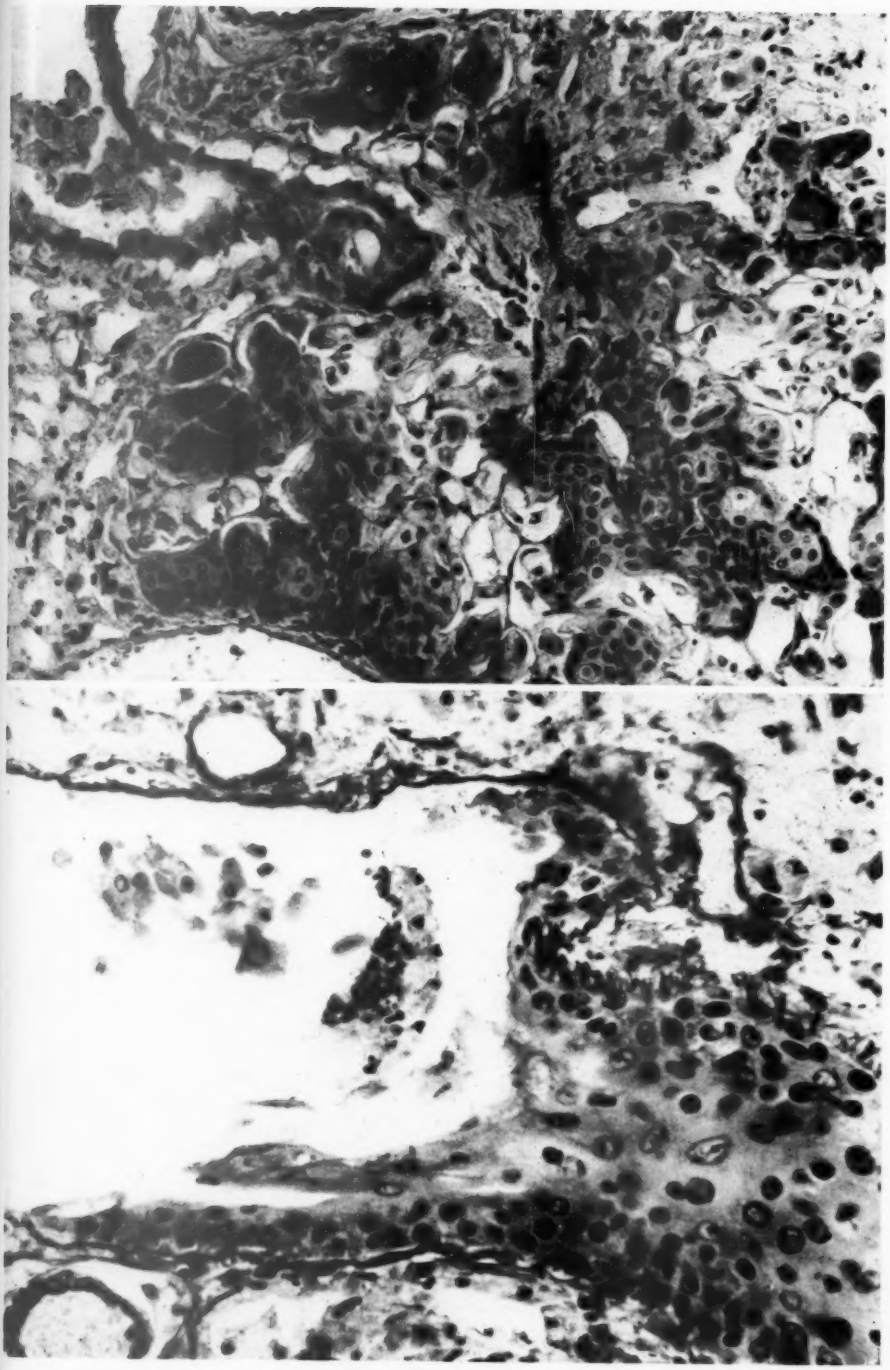

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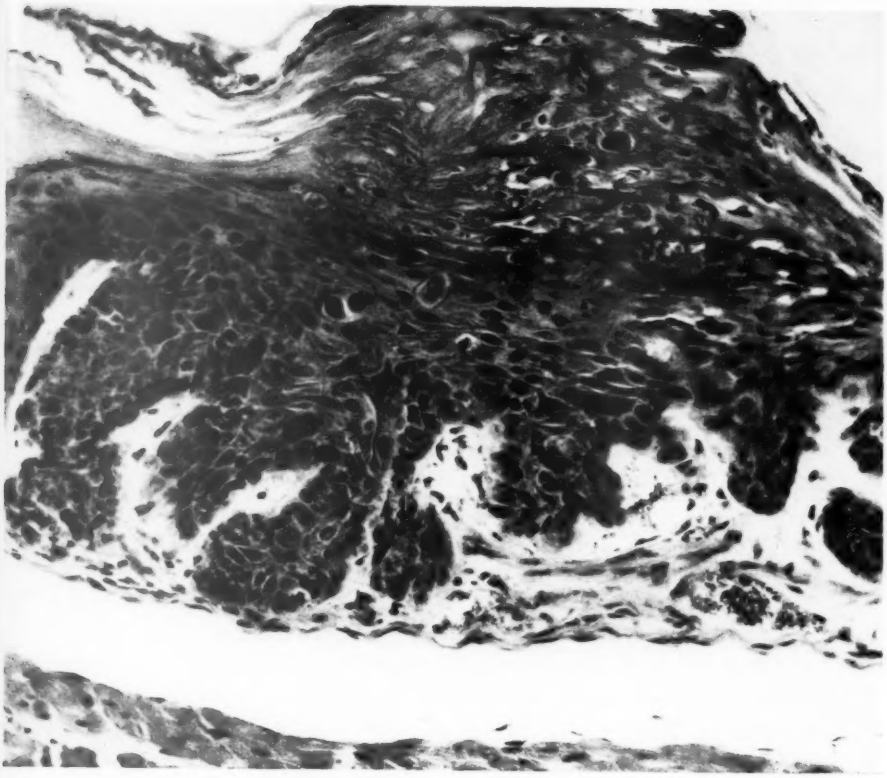
FIG. 8. Epidermoid carcinoma of the esophagus. The esophagus received 70,000 rem over a period of 237 days. × 250.



8

BRUCELLOSIS AND HEART DISEASE

II. Fatal Brucellosis: A Review of the Literature and Report of New Cases

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In 1941 the senior author encountered at necropsy a case of active brucellosis complicated terminally by signs of aortic valve disease (case 9). Acute endocarditis was suspected clinically; however, the necropsy examination revealed an essentially chronic lesion of the aortic valve. Was this the usual form of brucellar endocarditis? It was not possible to find the answer in standard texts since proved cases are rare. When the periodical literature on brucellar endocarditis was explored, the findings were of such interest and the implications appeared to be of such significance that the investigation was expanded to a review of the world literature on fatal brucellosis.

For this study we have read in the original all the case records we could find purporting to be instances of fatal brucellosis. In a few instances, reports not available in the original are quoted from another source; these are clearly designated in the references. Added to these are a number of previously unpublished cases from several sources, chiefly from the files of the Armed Forces Institute of Pathology; these new cases are reported briefly in the appendix.

All cases have been assigned to one of the following groups:

Group 1. Fatal *Brucella abortus* infection, with adequate necropsy data.

Group 2. Fatal *Brucella melitensis* infection, with adequate necropsy data.

Group 3. Fatal *Brucella suis* infection, with adequate necropsy data.

Group 4. Fatal Brucella infection, exact strain uncertain, with adequate necropsy data.

Group 5. Fatal probable brucellosis, with adequate necropsy data.

Group 6. Cases reported as fatal brucellosis, not included in other groups.

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TABLE I

FATAL *Brucella abortus* INFECTIONS, WITH ADEQUATE NECROPSY DATA (GROUP 1)

Case	Reported by	Origin	Age at death (yr.)	Sex	Duration of symptoms (mo.)	Previous carditis	Endocarditis	Embolic phenomena	Valves affected	Valve lesion	Myocardial aneurysm or abscess	Other myocardial lesions	Mode of death	Comments
1	Matzdorff[2]	Germany	37	M	4 (?)	"Heart trouble"	Yes	Kidney	Aortic	Hyaline	Yes	Resembled Aschoff bodies	Febrile state	
2	Steyrer[3]	Austria	28	M	11	No	Yes	Kidney, spleen	Aortic	Not described	No		Cardiac	Perforated cusps; pericarditis
3	Rothmann[4]	Germany	61	M	3	No	Yes	Spleen	Aortic	Not calcified	No	Granulomas	Cardiac	Granulomas, liver and kidney
4	Rennie & Young[5]	Great Britain	47	M	4	No	Yes	Spleen, liver	Mitral	Calcified	No		Not stated	
5	Sprunt & McBryde[6]	North Carolina	4	M	25		No				No		*Staph. aureus* bacteremia	Aplastic anemia; cellulitis of neck
6	Smith & Curtis[7]	Michigan	47	M	6	"Heart disease"	Yes	Petechiae	Aortic, tricuspid	Calcified	Yes		Febrile state	Large cells in endocardium resembling "Aschoff cells"
7	Spink & Nelson[8]	Minnesota	29	M	8	No	Yes	Petechiae	Aortic	Calcified	Yes	Granulomas	? Cardiac	Cardiac fluoroscopy suggested aortic stenosis
8	Spink Titrud & Kabler[9]	Minnesota	36	M	4	"Heart leakage"	Yes	Spleen, brain	Aortic, mitral	Calcified	No	Resembled Aschoff bodies	Cardiac	Perforated valve
9	Wechsler & Gustafson[10]	New York (or So. America?)	28	M	3 (or 144?)	"Heart attack"	Yes	Spleen; petechiae	Aortic	Hyaline	Yes		Febrile state	"Congenital bicuspid aortic valve"; perforated valve
10	Call et	Minnesota	27	M	2 (or	Rheumatic	Yes	No	Aortic	Calcified	Yes		Cardiac,	

10	Call et al.[11]	Minnesota	27	M	2 (or 90?)	Rheumatic fever	Yes	No	Aortic	Calcified	Yes		Cardiac, febrile	
11	Ibid	Minnesota	49	M	12 (or 168?)	Polyarthritis, fever	Yes	Petechiae	Aortic, mitral	Calcified	No		Cardiac	
12	Quintin & Stalker[12]	Canada	26	M	11	? Rheumatic fever	Yes	Spleen, ? brain; petechiae	Aortic, mitral	Calcified	Yes		Cardiac, embolism	Perforated valve
13	Voth[13]	Kansas	54	M	6	"Heart disease"	Yes	Petechiae; spleen, kidney	Aortic, mitral	Calcified	Yes	Granulomas	Cardiac, shock	
14	Hart, Morgan & Lacey[14]	Great Britain	48	M	17	"Bad heart"	Yes	Spleen, kidney	Aortic	Calcified	Yes		Cardiac	Pericardial adhesions; perforated valve
15	Purriel et al.[15]	Uruguay	adult	M	4	No	Yes	No	Aortic	Sclerotic	No	Granulomas	Cardiac	Pulmonary infarcts (?)
16	Spink[16]	Minnesota	33	M	32	No	Yes	Kidney, spleen	Aortic	Large friable vegetations	No		Not stated	
17	Grant & Stote[17]	England	51	M	6	No	Yes	No	Aortic	Bicuspid, calcified	Yes 2	Focal myocarditis	Cardiac tamponade	Ruptured cardiac aneurysm
18	Peery & Belter	Maryland	63	M	15	Dyspnea	Yes	Petechiae; spleen, heart, brain	Aortic, mitral	Calcified	Yes	Indistinguishable from Aschoff bodies	Embolic (?)	
19	Peery & Belter	Tennessee	54	M	30	No	Yes	No	Aortic	Calcified	No	Resembled Aschoff bodies	Cardiac	Portal cirrhosis
20	Peery & Belter	Oregon	59	M	2½ (or 86?)	No	Yes	Spleen	Aortic	Calcified	Yes	Interstitial fibrosis	Sudden collapse	Thrombosis or embolism, coronary artery

Those cases in which the diagnosis was culturally proved and confirmed by necropsy (groups 1 to 4) have been separated from the other cases and serve as the basis for our conclusions. The paucity of reported cases is somewhat surprising. This is undoubtedly due to a combination of factors: knowledge of brucellosis is almost confined to the last 60 years[1]; the diagnosis of brucellosis is difficult to establish; and few patients die of brucellosis.

Summary of Necropsy Observations in Fatal Brucellosis

Including the new cases described in this report, we have found 20 culturally proved examples of fatal infection due to *Brucella abortus* in which necropsy data are adequate (group 1; Table I). The age distribution is as follows: 4 years, 1 case; 21 to 40 years, 8 cases; 41 to 60 years, 8 cases; 61 years or older, 2 cases. All patients in this group were males. The average duration of symptoms was about 9 months. All except one showed evidence of endocarditis. The sole exception is the case reported by Sprunt and McBryde[6]; death in this 4-year-old boy should, perhaps, be ascribed to aplastic anemia and staphylococcus cellulitis of the neck, but the authors reported the recovery of *Brucella abortus* from postmortem cultures of the spleen and liver. In 15 instances there was necropsy evidence of embolism from the vegetations, but in only 7 were petechiae noted, either during life or at necropsy. Among the 19 cases showing endocarditis, the aortic valve was involved in 18 instances, the mitral valve in 6, and the tricuspid valve in 1. There was a striking tendency toward calcification of the affected valve, this having been specifically noted in 13 cases (68 per cent); in 3 other instances, the valve was described as hyaline or sclerotic. There was perforation of a valve cusp in 5 cases. In 11, abscesses or aneurysms of the myocardium were noted, usually continuous with the valvular vegetation. Other inflammatory lesions of the heart were noted in 10 cases; in 4 of these the authors noted the resemblance of the myocardial lesions to the Aschoff bodies described in rheumatic fever. In 2 instances there was involvement of the pericardium. Although granulomatous lesions were occasionally noted in other organs in this group, the serious lesions were confined largely to the heart. In at least 11 cases, death was thought to be a result of cardiac failure or decompensation.

There are 13 examples of fatal *Brucella melitensis* infection, proved by culture, in which the necropsy record was adequate (group 2; Table II). The youngest patient was 15 years old; the remainder were 28 to 54 years of age. Ten were males and 3 were females. The average duration of symptoms was about 6 months. In the 13 cases there are 9 instances of endocarditis. The exceptions were a 15-year-old boy reported

by Amuchastegui and Herrero[23] and 3 females reported by Arias.[25] In 8 of the 9 cases of endocarditis there was necropsy evidence of embolism from the valvular vegetations, and petechiae were observed in 6 cases. The affected valve cusps were perforated in 4 instances. The infectious process involved the aortic valve in 5 cases, the mitral valve in 4, and the pulmonic valve in 1. Again there was a tendency toward calcification and healing in the valve lesions; in 2 instances calcium deposits were specifically mentioned, and in 5 others the lesions were described as old, hyaline, sclerotic, or thickened. In 2 cases there was an abscess in the myocardium. In 5 instances cellular infiltration of the myocardium was described; in one of these a resemblance to Aschoff bodies was noted. There was pericardial involvement in 4 cases. In one instance death was due to massive peritoneal hemorrhage, presumably from a mycotic aneurysm, although none was found. The 3 cases reported by Arias,[25] all females, were said to show hepatitis; in 1 additional case there was evidence of cirrhosis of the liver. Inflammation of the brain and meninges was described in 2 cases. In 6 instances, death was ascribed to cardiac failure.

Only 7 cases of fatal *Brucella suis* infection, proved by culture, could be found for inclusion in group 3 (Table III). All of these were from the United States. They were males ranging in age from 21 to 62 years. In 2 the illness was a brief septicemia with no particular organ localization; in the 5 other cases symptoms were present for 4 to 84 months. Valvular heart lesions were noted in 3 cases, and in 2 of these the valve cusps were perforated. Embolism from the vegetations occurred in 2 of the 3 cases of endocarditis; petechiae were observed in none. The aortic valve and the mitral valve were each involved twice by the infectious process. Calcification in the valve cusps was not specifically described, but in 2 cases the valvular lesions were noted to be fibrotic. An abscess of the myocardium was encountered in 1 case. Myocardial lesions were noted in only 1 case.[31] In 2 cases, only 1 of which was described as showing endocarditis, death was due to rupture of an arterial aneurysm (basilar, femoral). In 1 case death was due to cardiac decompensation.

There are 4 cases in group 4 (Table IV), in which the Brucella was cultured but was not identified as to strain. In each there was adequate necropsy data. The ages ranged from 21 to 54 years. Again all cases were males. The duration of symptoms was somewhat uncertain. Endocarditis was present in all 4. Embolic phenomena occurred in all with the possible exception of case 4. Petechiae were noted in 3 cases. The aortic valve was affected in every instance, the mitral valve in 3. The valve cusps were perforated in 2 cases. In 1 case the valve cusps were described as calcified; in 2 others they were hard or rigid. An aneurysm

TABLE II

FATAL *Brucella melitensis* INFECTIONS, WITH ADEQUATE NECROPSY DATA (GROUP 2)

Case	Reported by	Origin	Age at death (yr.)	Sex	Duration of symptoms (mo.)	Previous carditis	Endocarditis	Embolic phenomena	Valves affected	Valve lesion	Myocardial aneurysm or abscess	Other myocardial lesions	Mode of death	Comments
1	De la Chappelle[18]	Italy or New York (?)	38	M	7	No	Yes	Petechiae; spleen, toe	Aortic	Hyaline	No		Hemorrhage	Pericardial effusion; hemoperitoneum (from mycotic aneurysm?)
2	Casanova & d'Ignazio[19]	Italy	28	M	9	No	Yes	Petechiae; spleen, kidney	Aortic	Old	No		Cardiac	
3	Silbergleit[20]	Italy	30	M	2	No	Yes	Lungs	Mitral, pulmonic	Not stated	No		Cardiac	Pericardial effusion; perforated valve
4	Levy & Singerman[21]	Vermont or Connecticut (?)	54	M	2½	Dyspnea	Yes	Petechiae; spleen, kidney	Mitral	Fibrous	No		Febrile state	Jaundice
5	Posteli et al.[22]	Italy	54	M	2	No	Yes	Petechiae	Aortic	Not stated	No		Cardiac	Perforated cusp; cirrhosis

6	Amuchastegui & Herrero[33]	Argentina	15	M	14		No				No	Interstitial inflammation	Febrile state	Bronchiectasis; spleen 1,140 gm.
7	Ibid	Argentina	42	M	?14	No	Yes	Spleen, kidney	Aortic	Calcified	Yes	Interstitial inflammation	Febrile, cardiac	Pericarditis; perforated cusp
8	Beebe & Meneely[34]	New York	31	M	12	Rheumatic fever	Yes	Petechiae	Aortic	Sclerotic	No		Febrile state	Pericarditis; perforated cusp
9	Arias[35]	Peru	47	F	?2		No				No	Interstitial inflammation	Hepatic coma	Subacute atrophy of liver; meningoencephalitis
10	Ibid	Peru	30	F	½		No				No		Febrile state	"Brucellotic hepatitis"; pneumonia
11	Ibid	Peru	34	F	?2		No				No	Interstitial inflammation	Febrile state	"Brucellotic hepatitis, meningitis"
12	Peery & Belter	Texas	48	M	10	No	Yes	Petechiae; spleen, kidney	Mitral	Calcified	No	Granulomas, some resembling Aschoff bodies	Cardiac	Pneumonia; pulmonary infarcts
13	Peery & Belter	Texas & Mexico	43	M	4	No	Yes		Mitral	Thickened	Yes		Febrile, cardiac	Jaundice, sulfa therapy

TABLE III

FATAL *Brucella suis* INFECTIONS, WITH ADEQUATE NECROPSY DATA (GROUP 3)

Case	Reported by	Origin	Age at death (yr.)	Sex	Duration of symptoms (mo.)	Previous carditis	Endocarditis	Embolic phenomena	Valves affected	Valve lesion	Myocardial aneurysm or abscess	Other myocardial lesions	Mode of death	Comments
1	Hardy et al.[26]	Iowa	21	M	4	No	Yes	Brain	Aortic	Not stated	Yes		Cardiac	Valve cusps destroyed
2	Hansmann & Schenken[27]	Iowa	24	M	11		No				No		CNS hemorrhage	Ruptured aneurysm, basilar artery; meningo-encephalitis
3	Menafee & Poston[28]	North Carolina	26	M	25		No				No		Febrile state	Jaundice; granulomas, parenchymatous organs
4	De Gowin et al.[29]	Iowa	45	M	10	No	Yes	Spleen	Mitral	Fibrosis	No		Hemorrhage	Ruptured aneurysm, femoral artery; ruptured valve cusp; nephritis
5	Meyer[30]	California	24	M	1/3		No				No		Febrile state	Micro-abscesses in various organs
6	Lowbeer[31]	Oklahoma	62	M	84	No	Yes		Mitral, aortic	Fibrotic	No	Scattered areas of fibrosis	Febrile state	Osteomyelitis, hip and pelvis
7	Peery & Belter	North Carolina	60	M	1/3		No				No		Febrile state	

TABLE IV

FATAL BRUCELLA INFECTIONS, EXACT STRAIN UNCERTAIN, WITH ADEQUATE NECROPSY DATA (GROUP 4)

Case	Reported by	Origin	Age at death (yr.)	Sex	Duration of symptoms (mo.)	Previous carditis	Endocarditis	Embolic phenomena	Valves affected	Valve lesion	Myocardial aneurysm or abscess	Other myocardial lesions	Mode of death	Comments
1	Scott & Saphir[22]	Ohio	21	M	10	Rheumatic fever	Yes	Petechiae; spleen, brain (?)	Aortic, mitral	Rigid; acute and chronic	No	Interstitial inflammation	Febrile state	Pericardial adhesions
2	Puech et al.[23]	France	25	M	3 (or 39?)	"Articular rheumatism"	Yes	Petechiae	Aortic, mitral	Hard vegetations	No	Interstitial inflammation	Cardiac	Pericarditis; perforated valve cusps; uremia
3	Gounelle & Warter[24]	Tunisia	45	M	3	No	Yes	Petechiae; kidney	Aortic	Ulcerative	Yes		Cardiac	Perforated valve cusps; pericarditis
4	Peery & Belter	Massachusetts	54	M	2 (or 11?)	Heart murmur	Yes	Heart (?)	Aortic, mitral	Calcified	No	Old infarct, granulomas like Aschoff bodies	Febrile state	Coronary thrombosis (embolism?)

of the myocardium was noted in 1. Two others showed evidence of myocarditis, and in the fourth there was an old myocardial infarct, possibly embolic in origin. There was pericardial involvement in 3 cases. In 2 instances, death was ascribed to cardiac failure.

Group 5 comprises 15 cases (Table V) reported as fatal brucellosis, where the diagnosis was based on a serum agglutination titer of 1:500 or above, rather than on culture. In some of these cases cultures were negative; in others they were not done. In all other respects these cases were satisfactory for evaluation. The youngest patient was 19 years of age. The remainder ranged from 24 to 67 years. All but 2 were males. The average duration of symptoms was approximately 13 months. Endocarditis was described in 10 of the 15 cases, and in 3 the valve cusps were said to be eroded or perforated. Embolism from the vegetations occurred in 9 instances, but petechiae were noted in only 2. The aortic valve was involved in 7 instances, the mitral in 4; in 1 case the affected valve was not named. There was a specific description of calcification of the valve cusps in 2 cases; in 5 others the valves were described as gritty, fibrotic, sclerotic or hyaline. An aneurysm of the myocardium was noted in 1 case, and other myocardial lesions were described in 6 cases. In 2 of these a resemblance to Aschoff bodies was noted; in a third the lesions were like Bracht-Wächter bodies. In 5 instances there was pericarditis. In 10, death was apparently a result of cardiac failure; in 2 there was myocarditis but no endocarditis.

Group 6 is composed of those cases considered by their authors to be instances of fatal brucellosis but excluded from the other groups for one reason or another. In some the diagnosis of brucellosis is considered uncertain or even unlikely; in others it appears that some disease unrelated to brucellosis was the cause of death; in many there was either no necropsy or the description of the necropsy was inadequate for our purposes. These 86 cases * are not considered further in this analysis.

Discussion

There are, then, 44 cases in groups 1 to 4 in which the diagnosis of brucellosis was established by culture. In each, death was a result of the infection or of complications therefrom, and an adequate necropsy report was available. In these cases there was some form of carditis in 38 instances (86 per cent). Endocarditis was by far the most important manifestation, being present in 35 instances (80 per cent). The incidence of endocarditis was highest in fatal infections due to *Brucella*

* References 44 to 63 cite most of the cases in group 6. The following authors, some of whose cases have been listed in other groups, are also represented in group 6: Hardy and colleagues,[50] 9 cases; Curschmann,[57] 2 cases; Lowbeer,[61] 3 cases; Arias,[58] 3 cases.

abortus (95 per cent). In the *Brucella melitensis* group, endocarditis was found in 69 per cent, and in the *Brucella suis* group, in 43 per cent.

The endocardial lesions of brucellosis appear to be a result of direct invasion of the valves by the infecting organisms. The micro-abscesses within the valve cusps, the destruction of the commissures, and the nodular, calcific nature of the deformity indicate that this is a chronic bacterial endocarditis. There was nothing about the lesions to suggest a hypersensitivity reaction; eosinophils were lacking and there was no evidence of arteritis.

Brucellar endocarditis may apparently develop on previously normal valves, if this can be inferred by the absence of cardiac symptoms and signs before the onset of brucellosis. Among the 35 cases of brucellar endocarditis in the culturally proved group, there was a prior history of rheumatic fever, heart disease, or dyspnea in only 15 (43 per cent). In the patients in whom there was such a history, it was often impossible to determine whether the symptoms were actually due to previous rheumatic fever or to earlier attacks of brucellosis.

There is a specific but not exclusive affinity of the Brucella for the aortic valve. Among the 35 instances of endocarditis at necropsy, the aortic valve was involved in 29 (83 per cent). The mitral valve was affected in 15 (45 per cent). The affinity for the aortic valve was particularly remarkable among the infections due to *Brucella abortus;* in this group the aortic valve was involved in 95 per cent of the cases showing endocarditis, and the mitral valve in 32 per cent.

Another noteworthy feature of the endocarditis due to Brucella is the frequency of calcification of the affected cusps and of the vegetations themselves. Among the 35 cases of endocarditis, calcification was specifically mentioned in 16 (46 per cent). This feature was particularly common in endocarditis due to *Brucella abortus,* in which calcification was specifically noted in 13 cases (68 per cent). Calcification has also been noted in the lesions of brucellosis in other organs, in both experimental animals and in man.[31,64–66]

Embolism and infarction stemming from valve vegetations seem to be less frequent in brucellar endocarditis than in that due to other organisms. There was necropsy evidence of embolism of the internal organs in 63 per cent of the 19 cases of endocarditis due to *Brucella abortus,* and of petechiae of the skin in 37 per cent. Focal embolic glomerulonephritis was extremely uncommon.

A remarkable feature of fatal brucellar endocarditis is that, at least in these cases, it is exclusively a disease of males (100 per cent).

Patients dying of brucellar endocarditis are older on the average than those who die with other forms of bacterial endocarditis. The cases

TABLE V

FATAL PROBABLE BRUCELLOSIS, WITH ADEQUATE NECROPSY DATA (GROUP 5)

Case	Reported by	Origin	Age at death (yr.)	Sex	Duration of symptoms (mo.)	Previous carditis	Agglutination	Endocarditis	Embolic phenomena	Valves affected	Valve lesion	Myocardial aneurysm or abscess	Other myocardial lesions	Mode of death	Comments
1	Ivarsson[35]	Denmark	42	M	2		1:5000	No				No	No	Hemorrhage	Thrombosis, femoral veins; intestinal hemorrhage
2	Ebskov & Harpøth[36]	Denmark	19	M	7+	No	1:1600	Yes	No	Aortic	Not stated	No	Not stated	Cardiac	Anemia; nephritis; pericarditis
3	Curschmann[37]	Germany	65	M	2		1:1600	No				No	Subacute myocarditis	Cardiac	Purulent pericarditis; pulmonary emboli
4	Wohlwill[38]	Germany	67	F	1		1:3200	No				No	No	Sudden	Pulmonary emboli
5	Harkness[39]	Great Britain	32	M	32	No	1:784	Yes	Petechiae; kidney, spleen	Mitral, aortic	"Gritty," fibrotic	No	Not stated	Cardiac	
6	Werthemann[40]	Switzerland	34	M	5	Heart murmurs	1:800	Yes	Spleen	Aortic	Calcified	No	Myocarditis	Cardiac	Pericarditis; encephalomeningitis

7	Raynaud et al.[41]	Algiers	50	M	4	No	1:500	Yes	Spleen, kidney	Aortic	Fibrotic	No	Not stated	Uremia	Spleen, 1,300 gm.
8	Spivak & Cerepnina[42]	Russia	30	F	24 (?)	No	1:1600	Yes	Spleen, kidney	Not stated	Not stated	No	"Ulcerating myocarditis"	Cardiac	Pericarditis; periarteritis
9	Stigliani[43]	Italy	26	M	72	No	1:1500	Yes	Spleen, brain	Mitral	Sclerotic	No	Bracht-Wächter bodies	Not stated	
10	Ibid	Italy	46	M	?	No	1:2000	Yes	Brain	Aortic	Not fibrotic	No	Not stated	Cardiac	Cusp eroded
11	Peery & Belter	Illinois (or Great Britain?)	25	M	7		1:10000	No				No	Like Aschoff bodies	Cardiac	Thrombophlebitis, mural thrombi
12	Peery & Belter	Virginia	51	M	18	No	1:10240	Yes	Spleen, kidney, mesentery	Aortic	Hyaline	No	No	Febrile, cardiac	Pulmonary infarct, ruptured aortic cusp
13	Peery & Belter	Virginia	30	M	8	No	1:1280	Yes	Petechiae; spleen, pancreas, mesentery	Mitral, aortic	Fibrotic	Yes	Like Aschoff bodies	Cardiac	Pericarditis, ruptured cusp, lung infarcts
14	Peery & Belter	California	29	M	2	Heart disease	1:1280	Yes	Spleen; petechiae	Mitral	Calcified	No	Perivascular infiltration	Febrile, cardiac	Pneumonia
15	Peery & Belter	Iowa	24	M	6		1:1280	No				No	No	Pulmonary embolism	Pulmonary infarcts, granulomas of liver and spleen

were divided as follows among the decades: 21 to 30 years, 10 cases; 31 to 40 years, 5 cases; 41 to 50 years, 9 cases; 51 to 60 years, 7 cases; 61 years or older, 3 cases; one case was designated simply as "adult."

The common mode of death in brucellar endocarditis is congestive heart failure rather than embolism or sepsis. Heart failure was noted in 60 per cent of all the cases of endocarditis.

Gross abscesses of the myocardium, or their equivalent, aneurysms, are apparently more frequent in brucellar endocarditis than in endocarditis due to other bacteria. This may well be correlated with the selective involvement of the aortic valve; abscesses are apparently more frequent in endocarditis of the semilunar valves than in that affecting atrioventricular valves. Abscesses occurred in 43 per cent of the cases with brucellar endocarditis, and in 58 per cent of those due to *Brucella abortus*.

Other inflammatory lesions of the myocardium were commonly encountered in fatal brucellosis. These lesions were focal, microscopic in size, paravascular, usually granulomatous and fibrotic, and often resembled Aschoff bodies. In 6 of the 12 fatal cases we have studied personally, myocardial lesions resembling Aschoff bodies were encountered. This can be compared with the incidence of Aschoff bodies in fatal rheumatic fever (32 to 87.5 per cent) as reported by various authors cited by Clawson.[67]

Brucellar endocarditis closely resembles calcareous disease of the aortic valve. Both show a remarkable prevalence in mature males. Brucellar endocarditis attacks the aortic valve selectively and produces a nodular, deforming lesion with a striking tendency to calcification. It is possible that the common valvular lesion, calcific aortic stenosis, believed by many to be a form of rheumatic heart disease, may occasionally be a sequel of brucellar endocarditis. For such a hypothesis to be considered, it must first be shown that endocarditis due to Brucella is not always fatal but may heal spontaneously, leaving a residual valvular lesion; this appears to be the case.[68] Analysis of other pertinent data suggests that brucellosis may in fact be the chief cause of calcific aortic stenosis.[69]

The observations cited would appear to justify the following conclusions:

1. The usual cause of death during brucellosis is endocarditis. In some instances myocarditis and pericarditis are evident.

2. Fatal Brucella endocarditis is essentially a chronic disease, with a greater tendency toward fibrosis, hyalinization and calcification than is usually noted in endocarditis caused by other bacteria. Because of these

features, most patients die from valve deformity and congestive heart failure rather than from sepsis or embolism.

3. The disease chiefly affects the aortic valve. In infections due to *Brucella abortus*, the strain most often encountered in the United States, this valve is involved more than twice as frequently as all other valves combined. The reason for this affinity is not apparent.

4. A prevalence in males is probably attributable to the fact that serious brucellar cardiac lesions are usually a result of heavy, recurrent occupational exposure, as in farmers, meat packers and veterinarians.

5. The disease tends to occur in middle adult life. In *Brucella abortus* infection, the average age at death from endocarditis was 43 years. This late development may be due in part to a natural immunity in children and adolescents.[70] It may also mean that repeated infection over many years is necessary for the development of the endocarditis.

6. Lesions resembling Aschoff bodies are commonly found in the myocardium in fatal brucellosis. Even when there are no "typical" Aschoff bodies—and this is primarily a matter of individual interpretation—it is common to encounter focal accumulations of lymphocytes and mononuclear cells together with patchy areas of interstitial fibrosis.

7. The observation of chronic valvular heart disease and "Aschoff bodies" in fatal infections due to Brucella makes it imperative that "rheumatic fever" be re-evaluated. "Rheumatic fever" may well be a syndrome including several diseases having febrile episodes and a tendency to involve the joints and the heart.[71]

Appendix: Report of New Cases

Fatal Brucella Abortus Infections (Group 1)

Case 1. (A.F.I.P. #569444; case 18, Table I.) A 63-year-old white male was ill for about 15 months with irregular fever and recurring chills. There was no history of contact with farm animals, drinking raw milk, or rheumatic fever. Physical examination disclosed evidence of stenosis and insufficiency of the aortic valve. The liver and spleen were readily palpated. A blood culture taken 3 weeks before death was positive for Brucella. The VDRL and Kolmer tests were negative; a Kahn test was 4 plus (16 units).

Death occurred unexpectedly 10 days after admission. The clinical impression was subacute bacterial endocarditis, with embolism, possibly to the coronary arteries.

Necropsy was performed by Dr. Chapman H. Binford at the United States Marine Hospital, Baltimore, Maryland. The heart weighed 620 gm., the hypertrophy being chiefly of the left ventricle. There was an old infarct of the left ventricular myocardium near the apex. The mitral valve showed some thickening of the base of the aortic cusp. All of the cusps of the aortic valve were thickened and calcified, showing both old and recent vegetations. The posterior and the right anterior cusps were fused, and their common commissure was displaced downward. There was a perforation 1.5 cm. in diameter in the posterior cusp of the aortic valve. Opening into the aorta, lateral to this perforation, there was a 2 cm. aneurysm in the myocardium. Microscopic sections of the vegetations revealed extensive calcium deposits within dense hyaline connective tissue (Fig. 1). Some fields also showed foci of necrosis within the valve cusp and surface deposits of fibrin. Smears demonstrated numerous short gram-negative bacilli. Random sections of the myocardium ex-

hibited focal paravascular lesions containing stringy conglomerates of fibrinoid substance and large spindle-shaped and pleomorphic cells; these lesions were considered to be indistinguishable from the Aschoff bodies of rheumatic fever (Figs. 2 and 3). Cultures of heart blood, aortic valve vegetations, liver and spleen revealed *Brucella abortus*. There were infarcts in the spleen and brain.

Case 2. (A.F.I.P. #279996; case 19, Table I.) A 54-year-old white dairy farmer noted a sudden spell of dyspnea and precordial pain about 30 months before his death and remained in bed for 2 days. About 15 months later, chest pain, dyspnea and edema appeared, and there were fever, chills and sweats. A harsh systolic murmur was heard over the entire precordium. Terminally there was also a diastolic aortic murmur. Death was a result of heart failure. Blood cultures were repeatedly positive for *Brucella abortus* during the last 5 months of life.

At necropsy the heart weighed 500 gm. There were rough, calcified plaques on the leaflets of the aortic valve; one commissure was destroyed. Microscopic sections of the valve showed micro-abscesses surrounded by hyaline connective tissue containing calcium deposits (Fig. 4). The myocardium was the seat of focal paravascular monocytic infiltration (Fig. 5). The liver exhibited cirrhosis, but no noteworthy lesions were observed in the other organs.

Case 3. (A.F.I.P. #276412; case 20, Table I.) A 59-year-old white ranch foreman died suddenly after a 10-week period of chills, fever and weight loss. Systolic and diastolic murmurs were heard at the cardiac apex and over the lower sternum. Serologic tests for syphilis, negative 4 years previously, were positive terminally. A blood culture was positive for *Brucella abortus*. An electrocardiogram showed changes suggestive of a remote anteroseptal infarction.

At necropsy the heart weighed 900 gm. All of the valves except the aortic appeared normal. The aortic cusps were rigid, nodular and calcified; the valve was believed to be both stenotic and regurgitant. There was a 3 cm. aneurysm in the interventricular septum; this was thought to be a mycotic aneurysm of the left anterior descending coronary artery. The aorta showed gross and microscopic alterations characteristic of syphilitic aortitis. Microscopically, there was myocardial necrosis and a diffuse leukocytic infiltration in the region of an abscess. Adjacent branches of the coronary arteries were infiltrated with neutrophils, but no lesions resembling Aschoff nodules were noted. The spleen contained multiple infarcts, but the other organs, including the brain, were not remarkable.

Fatal Brucella Melitensis Infections (Group 2)

Case 4. (A.F.I.P. #58597; case 12, Table II.) A 48-year-old male had performed necropsies on a number of goats which had died of an unknown disease. His own illness lasted 10 months and was characterized by fever, chills, joint pains and weight loss. There was atrial fibrillation, but no murmurs were heard. The spleen was palpable. A blood culture yielded *Brucella melitensis*.

At necropsy, scattered petechiae were noted in the skin. The heart weighed 410 gm. Only the mitral valve was abnormal. This showed a mass of firm, grayish white endocardial vegetations which almost completely obliterated the valve opening. Microscopically, the vegetation was largely acellular and fibrinous, but it contained numerous calcium deposits (Fig. 6). There were focal cellular nodules in the myocardium and in the subendocardial zone of the left atrium (Fig. 7).

Cultures of the heart blood and spleen grew *Brucella melitensis*.

Case 5. (A.F.I.P. #64684; case 13, Table II.) A 43-year-old Mexican had a 4-month illness with dyspnea, edema, chills and fever. A systolic thrill and murmur were noted at the cardiac apex. A blood culture was negative.

At necropsy, the heart was about normal in size. The mitral valve was markedly thickened, somewhat soft, and showed an ulcerated hemorrhagic lesion on the atrial surface. Cultures taken from this area yielded *Brucella melitensis*. The available sections did not include the valvular lesion. A section of left ventricle showed only irregular fibrous scarring. Other organs contained no significant abnormality.

Fatal Brucella suis Infections (Group 3)

Case 6. (A.F.I.P. #288404; case 7, Table III.) A 60-year-old farmer was ill only 11 days with fever, weakness and headache. He was admitted in shock and died a few hours later.

At necropsy, the heart showed no abnormality. The spleen weighed 500 gm. and was quite soft. Postmortem cultures taken from the spleen yielded *Brucella suis*. The other necropsy findings were not significant.

Fatal Brucella Infections, Exact Strain Uncertain (Group 4)

Case 7. (A.F.I.P. #317214; case 4, Table IV.) A 54-year-old white dairy farmer complained of vague upper abdominal pain beginning 11 months before his death. Seven months later he had a "typical episode of myocardial infarction." For the last 2 months his illness was marked by chills and fever. Twenty years previously he had been told that he had a heart murmur. From age 42 to 48 years, he had suffered with recurrent migratory arthritis involving all the joints except the spine. There was atrial fibrillation, and systolic and mid-diastolic murmurs were heard in the third left interspace. A blood culture yielded Brucella; the strain was not identified. The patient died following a sudden period of unconsciousness.

At necropsy, the heart weighed 590 gm. The mitral valve was moderately stenotic, and the cusps were thick and calcified. All cusps of the aortic valve were thickened and calcified. The anterior descending branch of the left coronary artery was occluded by a fresh thrombus, and there was an old myocardial infarct near the apex of the left ventricle. Both coronary arteries exhibited extensive atheromatous changes. Random sections of the myocardium showed focal lesions resembling Aschoff bodies (Fig. 8). The other organs were not remarkable; there were no other infarcts.

Fatal Probable Brucellosis (Group 5)

Case 8. (A.F.I.P. #139068; case 11, Table V.) A 25-year-old soldier, a native of Illinois, had a febrile illness for 7 months. This developed while he was convalescing in England from a battle wound received one month earlier. At one time a blood serum agglutinated the Brucella antigen in a dilution of 1:10,000. Palpitation and weakness were conspicuous symptoms, but no murmurs were heard. Death was the result of heart failure.

At necropsy, the heart weighed 420 gm.; it was globular, flabby, and thrombi were adherent to the endocardium of both ventricles. The valves showed no lesions. Microscopically, the myocardium contained foci of intensive inflammation, and in some areas there were lesions resembling Aschoff bodies. Both femoral veins contained adherent thrombi which extended into the inferior vena cava. There were no pulmonary emboli, and the other organs showed only congestion. Postmortem cultures were negative.

Case 9. (Case 12, Table V.) A 51-year-old preacher-farmer had intermittent bouts of fever and arthritis for 18 months. A Brucella agglutination in the serum reached a peak of 1:10,240. Ten days before death, the patient developed severe anginal pain, dyspnea, and was found to have aortic insufficiency. Death was a result of heart failure.

At necropsy, the heart weighed 500 gm. At the left posterior commissure of the aortic valve there was a 1.5 cm., nodular, hyaline mass. The posterior cusp showed a ragged tear and a firm friable vegetation. The other cusps of the aortic valve were relatively normal, and the other valves were completely normal. Microscopically, there were no noteworthy lesions of the myocardium. Infarcts were noted in the lung, spleen and right kidney.

Case 10. (A.F.I.P. #613963; case 13, Table V. The records and slides on this case were made available through the kindness of Dr. Charles H. Lupton, formerly of the University of Virginia, now of the Department of Pathology at the University of Alabama.)

A 30-year-old farmer had an 8 months' illness marked by chills, fever, muscular aches, dyspnea and weakness. The titer for Brucella agglutinins in his blood rose during the illness from 1:160 to 1:1280. While he was under observation, systolic and diastolic murmurs appeared over the aortic area. The last few weeks of the illness were characterized by cardiac failure and petechiae.

At necropsy, the heart weighed 580 gm., and the epicardium was fibrin coated. The

tricuspid and pulmonic valves were normal. The mitral valve was somewhat thickened along its line of closure. The commissure between the right and the posterior aortic cusps was fibrotic, and there was an old rupture of the right aortic cusp near the commissure. In the sinus of Valsalva behind the ruptured cusp there was a 1 cm. aneurysm; on microscopic examination this was lined with endothelium. There were paravascular foci of mononuclear cells and lymphocytes simulating Aschoff nodules (Figs. 9 and 10). The valvular lesion was hyaline and fibrotic; there was no evidence of active necrosis or leukocytic exudate. There were evidences of an old embolism in several organs in the systemic circulation, and the lungs contained multiple infarcts.

Case 11. (Case 14, Table V. This case was first obtained from the files of the National Institutes of Health through the kindness of Dr. L. L. Ashburn; it is also case #26819 in the files of the Armed Forces Institute of Pathology.) This patient, a resident of California, was discharged from the United States Navy at age 22 years because of heart disease, the nature of which was not recorded. At 29 years he became ill with fever, precordial distress, dyspnea and cough. A presystolic thrill and rumble were noted at the cardiac apex. Agglutinins against Brucella were demonstrable in his blood at a titer of 1:1280. He died following a spell of dyspnea and violent coughing.

At necropsy, numerous petechiae were noted in the skin. The heart weighed 420 gm., and the right ventricle was hypertrophied. The mitral valve cusps were thickened and calcified; a large vegetation was present on both surfaces of its aortic cusp. The other valve orifices were apparently normal. Microscopic sections of the myocardium revealed perivascular and interstitial infiltration with lymphocytes and mononuclear cells. The only pertinent findings in other organs were 3 infarcts in the spleen.

Case 12. (A.F.I.P. #170106; case 15, Table V.) A 24-year-old white farmer-soldier had a 6 months' illness characterized by intermittent fever, cough and weakness, and terminally by chest pain and dyspnea. Examination of the heart was normal. Serum agglutination of Brucella was positive in a dilution of 1:1280.

At necropsy, the heart weighed 430 gm. but showed no abnormality, grossly or microscopically. The lungs contained recent infarcts and multiple massive emboli. The liver and spleen were enlarged and exhibited focal granulomas, but the other organs revealed no abnormalities.

Except as indicated, the new cases in the foregoing appendix are from the Armed Forces Institute of Pathology. The cooperation and encouragement of Dr. Chapman H. Binford, Chief of the Section on Infectious Diseases, Armed Forces Institute of Pathology, are gratefully acknowledged.

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LEGENDS FOR FIGURES

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

FIG. 1. A.F.I.P. #569444. Section of the aortic valve in a case of fatal endocarditis due to *Brucella abortus*. Large masses of calcium are noted in the free portion of the cusp (top) and at the base of the valve. Approximately × 20.

FIG. 2. A.F.I.P. #569444. Focal granulomatous lesions near small blood vessels in the myocardium of the left ventricle in fatal brucellar endocarditis. The lesion just to the left of center in the upper portion of the field is believed to meet the usual criteria for an "Aschoff body."* × 150.

FIG. 3. A.F.I.P. #569444. Another lesion resembling an "Aschoff body" in a fatal case of endocarditis due to *Brucella abortus*. Wavy bands of "fibrinoid necrosis" are seen in the center of a granulomatous lesion. A small artery is transected at the left of the field. × 300.

* The Aschoff body is a lesion about which pathologists argue at length and sometimes bitterly. Photomicrographs from these cases have been shown to a large number of pathologists, and each has identified one or more lesions which he believes "typical"—but the selections differ. Since judgment in this matter is necessarily based upon preconceived criteria that cannot be proved by experimental or other methods, perhaps one shouldn't be too dogmatic in his opinions!

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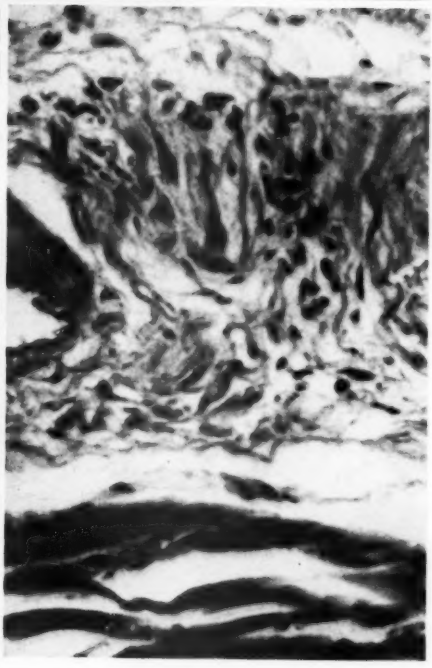

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FIG. 4. A.F.I.P. #279996. Section through a nodular aortic valve lesion in a fatal case of endocarditis due to *Brucella abortus*. Two micro-abscesses appear in dense inflammatory tissue; minute calcium deposits are evident on the left. Approximately × 60.

FIG. 5. A.F.I.P. #279996. A focal paravascular inflammatory lesion of the myocardium in fatal brucellar endocarditis. This lesion is comparable to the active "granulomatous phase of the Aschoff body," described by others. × 480.

FIG. 6. A.F.I.P. #58597. A mitral valve lesion in a fatal case of *Brucella melitensis* endocarditis. The surface portion of the vegetation shows a cellular infiltrate, but the deeper portion is largely acellular and hyaline. Calcium deposits are noted in the lower right portion of the field. × 80.

FIG. 7. A.F.I.P. #58597. A focal granuloma of the myocardium in a case of *Brucella melitensis* endocarditis. × 150.

FIG. 8. A.F.I.P. #317214. A focal paravascular granuloma resembling an "Aschoff nodule" in the myocardium in a case of brucellar endocarditis (strain not identified). × 150.

FIG. 9. A.F.I.P. #613963. Focal paravascular lesions resembling "Aschoff bodies" in the myocardium in a case of endocarditis probably due to Brucella; cultures were negative, but the agglutination titer rose from 1:160 to 1:1280 under observation. × 60.

FIG. 10. A.F.I.P. #613963. Other myocardial lesions in a case of probable brucellar endocarditis. While the cellular infiltrate is somewhat diffuse, these lesions also resemble "Aschoff bodies." × 180.

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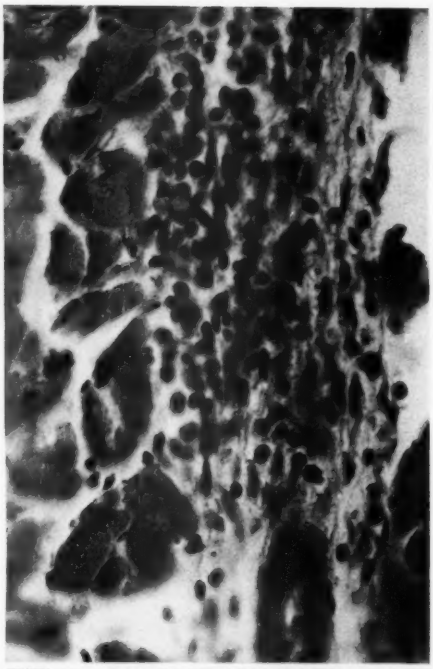

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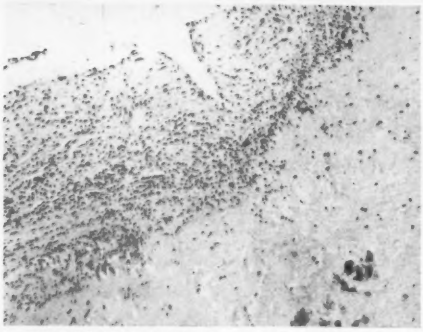

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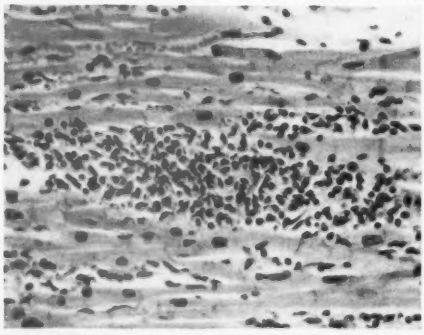

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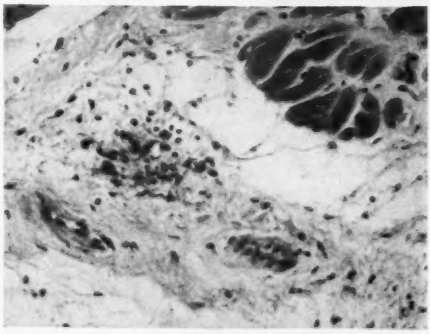

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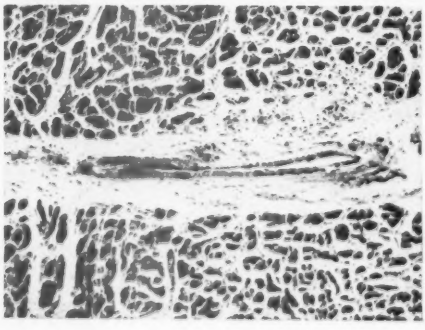

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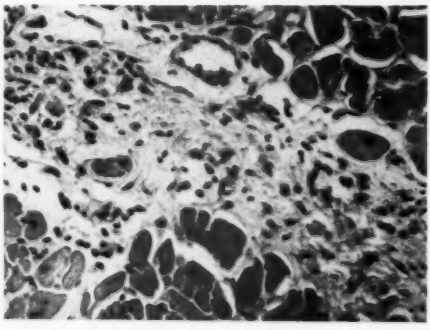

NEPHROTOXIC PROPERTIES OF COPPER UNDER EXPERIMENTAL CONDITIONS IN MICE

With Special Reference to the Pathogenesis of the Renal Alterations in Wilson's Disease

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Amino-aciduria, glycosuria, proteinuria, calciuria, and phosphaturia[1,2] have been observed frequently in cases of Wilson's disease (hepatolenticular degeneration). It is well known that this disorder is regularly associated with lowered concentrations of copper-binding globulin, ceruloplasmin, and copper in the serum, with deposition of the metal in many tissues, including the kidneys, and with hypercupruria.[3] Although it is also clear that copper has nephrotoxic properties,[4] these have not been defined precisely, and the possible role of copper itself in the pathogenesis of the aforementioned abnormalities remains obscure.[2,5]

Recent studies have shown that fish kept in water to which copper sulfate had been added took up and retained the metal within cells of many tissues. The concentrations in the kidneys equaled those in Wilson's disease, and conspicuous cytologic alterations appeared in the epithelium of the renal tubules.[6] In the present studies, copper has been given to mice in the form of a copper-albumin complex. Under these conditions the metal has accumulated in the kidneys, again in concentrations similar to those occurring naturally in patients with Wilson's disease, and marked necrosis of the epithelial cells of the proximal convoluted tubules has regularly been found. The observations as a whole make it seem likely that copper itself plays an important role in the pathogenesis of the renal abnormalities in hepatolenticular degeneration.

Material and Methods

Mice received intraperitoneal injections of a copper-albumin complex made by equilibrium dialysis. At regular intervals thereafter the concentrations of copper in various tissues were determined by chemical analysis; the distribution of the metal was studied by histochemical methods;

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and the cytologic effects were observed in tissues stained by histologic techniques and in individual nephrons dissected from digested specimens. To learn more about the manner of action of copper upon the renal tissues, the effects of reduced glomerular filtration upon the rate of accumulation of metal in the renal parenchyma was observed in animals with ligated ureters, and studies were made of the effects of repeated exposure of the renal epithelium to the metal.

Young adult mice of AKD_2/JAX strain, 20 to 30 gm. in weight, were used.

Copper-albumin Complex

A 5 per cent solution of bovine albumin (Armour) was made in demineralized water. In amounts of 300 cc., this solution was encased in cellulose tubing and placed in 3,000 cc. of a 0.0025 M copper sulfate solution (Mallinckrodt, analytical reagent grade) for approximately 6 hours or until the appearance of a fine, cloudy precipitate. Dialysis was then carried out in free-flowing tap water for 3 days. As proved by chemical analysis, one solution used in these studies contained 293 gamma of copper per cc. and another, 199 gamma.

Injection of Copper in Mice

Forty mice each received an intraperitoneal injection of 1.25 cc. of a solution of copper-albumin that contained 366 gamma of metal. As controls, 24 mice received 1.25 cc. of a 5 per cent solution of bovine albumin. Animals were sacrificed in pairs or groups of 4 at regular intervals from 1 to 144 hours later. In parallel studies 20 mice received injections of 368 gamma of copper in 1.85 cc. of a copper-albumin solution; each cc. contained 199 gamma of copper.

Ureteral Ligation and Copper Injection

The left ureters of 20 mice were doubly ligated just distal to the renal pelvis. Three days later 12 of the animals were given intraperitoneal injections of 366 gamma of copper in 1.25 cc. of a copper-albumin solution. With 8 mice serving as controls, pairs of animals were sacrificed periodically from 6 to 96 hours thereafter.

Multiple Injections of Copper in Mice

Thirty mice received intraperitoneal injections of 293 gamma of copper in 1 cc. of copper-albumin solution. Eight were sacrificed in pairs 6, 24, 48, and 96 hours later. Five days after the first injection, the remaining animals were given an injection of 293 gamma of copper, and 8

more were sacrificed in pairs 6, 24, 48, and 96 hours thereafter. Another 5 days after the second injection, the remaining animals received a third injection with 293 gamma of copper, and all animals were sacrificed in pairs 6, 24, 48, 72, and 96 hours later. The morphologic alterations in the kidneys were investigated.

Chemical Analysis for Copper

Tissues for chemical analysis were fixed in 10 per cent formalin made with demineralized water. Quantitative analyses for copper were performed upon duplicate samples by the method of Eden and Green, using the DU Beckman spectrophotometer.[7] The values were expressed in gamma of copper per 100 mg. of wet tissue.

Histologic and Histochemical Procedures

Routinely, small portions of tissue from all principal organs were fixed in 10 per cent formalin. These were embedded in paraffin, sectioned at 4 μ, and were then stained by hematoxylin and eosin, the periodic acid-Schiff reagent, the von Kossa method for phosphates to show calcification, the Perls method for iron, and histochemically for copper by rubeanic acid.[8]

Nephrons from both normal and experimental mice were dissected by the method of Oliver, MacDowell and Tracy.[9] Digestion by concentrated HCl required approximately 12 hours; the dissected nephrons were lightly stained with Mayer's hematoxylin.

Results

The Effects of Copper upon the Kidneys of Mice

Promptly after the intraperitoneal injection of copper-albumin, notable elevations occurred in the content of this metal in the liver, kidneys, spleen, and brain (Table I). Within one hour after the injection, copper in amounts of 2.19 gamma per 100 mg. of wet tissue were present in the renal parenchyma and persisted, for more than 6 hours, in concentrations that were 3 to 6 times normal. The content of copper then decreased precipitously and reached normal concentrations approximately 48 hours after the injection. The quantity of metal in the liver and spleen also increased markedly and remained elevated for more prolonged periods; in the neural tissues the content of copper was also notably elevated above the normal.

The kidneys appeared normal to gross examination up to 5 hours after the injection of copper-albumin. Within the next 2 hours notable enlargement of this organ occurred, and marked congestion of the cortex

became evident. Maximally enlarged kidneys, with weights up to 300 mg. (normal, 200 mg.), were present in animals sacrificed 24 and 48 hours after injection. The renal parenchyma was softened, and there was a sharp delineation between the blanched cortex and the dark congested medulla. Death occurred most often during this period, and regularly the kidneys were conspicuously altered. In contrast, the kidneys of animals that survived and were sacrificed 72 to 144 hours after the injection of copper either showed minimal degrees of congestion or were normal in appearance.

TABLE I

THE CONCENTRATION OF COPPER IN THE ORGANS OF MICE RECEIVING INTRAPERITONEAL INJECTIONS OF A COPPER-ALBUMIN COMPLEX

Hours after injection	Copper content, gamma per 100 mg. wet tissue				Copper content, gamma per 100 mg. wet tissue	
	Kidney	Liver	Spleen	Brain	Left kidney (ligated ureter)	Right kidney (patent ureter)
0	1.15	1.21	3.97	0.34	0.23	
controls	0.26	1.76	5.88	0.47	0.55	
controls	0.55	1.14	6.27	0.58	0.92	
controls	0.95	0.73	4.11	0.60		
controls	1.30	1.94	1.95	0.83		
1	2.19	3.37	7.93	—		
2	3.65	3.51	—	—		
3	4.17	3.65	6.88	—		
4	4.24	4.53	—	—		
5	3.71	3.70	5.06	—		
6	4.48	3.21	9.04	1.73	0.63	2.05
7	4.17	2.75	—	—		
24	1.26	2.68	10.40	1.72	0.63	2.09
48	0.74	2.39	10.65	2.82	0.53	0.35
72	0.45	1.65	4.45	2.33	0.25	0.75
96	0.16	2.25	7.21	2.83	0.49	0.56
120	—	1.41	6.88	1.99		
144	—	0.23	5.51	2.21		

The renal tissues taken 6 hours after the injection of copper-albumin, when stained histochemically for copper by rubeanic acid, showed it to be present in abundance and to stain as fine particles in the glomerular tufts and subcapsular spaces as well as in the content of the tubules. Small amounts were present in the epithelial cells of the proximal convoluted tubules, distributed haphazardly in the lumen margins of the cytoplasm (Fig. 1). More marked staining was regularly present in the epithelium 24 hours after the injection, the cells of the proximal convoluted segments and those of the distal conducting tubules staining most intensely (Fig. 2). At these times, the fine granules were distributed irregularly throughout the cytoplasm, but were not found within

the nuclei. In general, there was poor correlation between the quantity of copper that was stained by histochemical methods and the amounts shown to be present by chemical analysis.

The earliest cytologic lesions were clearly evident in the renal tissues taken 6 hours after the injection of copper-albumin. Initially, these were confined to the epithelium of the proximal convoluted segment of the nephrons and were characterized regularly by vacuolation, pale staining, and swelling of the cytoplasm. These alterations progressed rapidly to coagulative necrosis which varied moderately in degree from animal to animal but was ordinarily conspicuous in mice sacrificed 24 hours after the injection of copper. The cytologic changes remained localized to the proximal convoluted tubules. This was true even when they were marked in degree and characterized by lysis or hypereosinophilia of the cytoplasm, pyknosis and karyorrhexis of the nuclei, and extensive cellular desquamation. The tubules were filled with cellular debris. Many of the basement membranes were ruptured and erythrocytes were present in small numbers within the tubules (Fig. 3). Much granular basophilic material was also present in the cellular debris; this stained positively for phosphates and calcification, but did not stain for iron (Fig. 4). Examinations of the renal parenchyma obtained 72 to 144 hours after the injection of copper-albumin regularly showed striking degrees of epithelial regeneration, but with residual pleomorphism, hyperchromasia, and mitotic activity in the reconstituted lining epithelium of the proximal convoluted tubules. The glomeruli and renal blood vessels were unaltered.

Dissected Nephrons of Animals Injected with Copper-Albumin

The individual nephrons taken from mice 24 to 48 hours after the injection of copper-albumin and examined by light and phase microscopy consistently showed expansion of the proximal convoluted segments and obliteration of the normal epithelial architecture in this region. The delicate pattern of epithelial cells, clearly visible in the control specimens (Fig. 5), was replaced throughout the proximal convoluted segment by amorphous and coarsely granular masses of cellular debris. These filled the proximal convoluted tubules, and around them the basement membranes were swollen and fragmented. The diverticula that were numerous on normal nephrons were even more abundant and were notably dilated in animals that had received copper-albumin. The tubular dilatation and epithelial disruption ended abruptly just proximal to the thin loop of Henle (Fig. 6). The distal tubules were lined by epithelium and did not show significant dilatation, although many cellular casts lay within them. The glomeruli appeared normal.

The Effects of Reduced Glomerular Filtration Upon the Accumulation of Copper in the Kidneys

When copper-albumin was injected intraperitoneally into mice that had had the left ureter ligated previously, the content of copper in the left kidney increased only slightly and in amounts readily attributable to increases in the content of copper in the blood of the kidney. In contrast, significant elevations in the content of this metal occurred in the contralateral kidneys (Table I).

As shown in histologic sections, ligation of the ureter in control animals was always followed by dilatation of the tubules, which contained much fluid and were lined by atrophic epithelium. The injection of copper-albumin in such animals was not followed by additional cytologic alterations. Necrosis did not occur in kidneys with ligated ureters, but was regularly conspicuous in the contralateral ones where the histologic alterations were identical to those described above in animals without ureteral ligation.

The Effects of Repeated Injections of Copper-Albumin

The renal tissues taken from mice for histologic examination 24 and 48 hours after a single injection of 293 gamma of copper showed necrosis of the epithelium in the proximal convoluted segments of the nephrons. Identical lesions were present in mice examined at the same intervals after second and third injections of copper. Reconstitution of the renal epithelium had largely been completed in animals sacrificed 72 and 96 hours after the third injection. Variable quantities of calcium were present; generally these became greater after multiple injections.

Discussion

The observations make it clear that when copper was injected intraperitoneally in mice in the form of an albumin-complex, it passed promptly into the glomerular filtrate where, in sections stained histochemically for the metal, it was clearly visible in the subcapsular spaces of the glomeruli. As ancillary evidence, stoppage of glomerular filtration by ureteral ligation regularly excluded the metal from the renal parenchyma. It was also clear from the histochemical preparations that copper was then at least partially reabsorbed by the epithelium of the renal tubules, and when present in concentrations comparable to those in patients with hepatolenticular degeneration, as shown by chemical analysis, was accompanied by marked epithelial necrosis in the proximal convoluted tubules. These changes proved fatal in some animals. In others they were transitory, and epithelial regeneration occurred rapidly

but with residual epithelial hyperplasia and calcification. The findings make it evident that therapeutic measures designed to increase the excretion of copper by the kidney in Wilson's disease[10] are potentially hazardous.

It is well known that because persons with hepatolenticular degeneration have a deficiency of the copper-binding globulin, ceruloplasmin, the copper they take in through the gastrointestinal tract is loosely bound by serum albumin in the form of a copper-albumin complex.[8] As has been stated above, the molecular composition and the manner of transmission of the metal through the kidneys of human beings remains obscure,[2,5] and its cytophysiologic effects upon the renal tissues also remain in doubt.[3] Although the present studies provide direct information only about the cytotoxic action of copper upon the nephron, observations by others have provided much evidence to suggest that these cytologic alterations can be correlated directly with certain renal dysfunctions.[11] In this relation, it is also noteworthy that intoxications by other heavy metals, particularly by mercury, cadmium, uranium, and lead, provide additional examples of cytotoxic agents that cause similar cytologic changes in the epithelium of the proximal convoluted tubules, and these are frequently associated with amino-aciduria, proteinuria, and calciuria.[12,13]

Summary

When ionized copper was combined with bovine albumin and injected intraperitoneally into mice as a copper-albumin complex, it promptly entered the glomerular filtrate and was taken into the cytoplasm of the renal tubular epithelium. Marked necrosis of the proximal convoluted tubular epithelium occurred when concentrations of copper were present that approximated those frequently found in the kidneys of patients with hepatolenticular degeneration. Death ensued in some animals, while in others epithelial regeneration occurred promptly, with residual epithelial hyperplasia and calcification. The observations as a whole, when considered in the light of observations upon other nephrotoxic agents, make it seem likely that copper itself is an important factor in the pathogenesis of the renal abnormalities of Wilson's disease.

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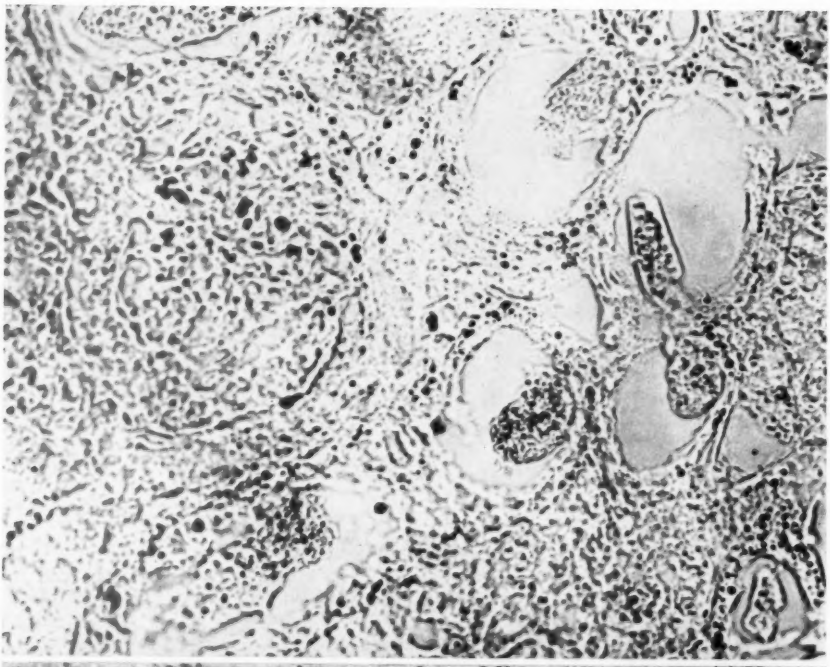
The proficient technical assistance of Miss Lieselotte Kemper is gratefully acknowledged.

LEGENDS FOR FIGURES

FIG. 1. A mouse received 366 gamma of copper intrapertioneally, as a copper-albumin complex. After 6 hours there is much metal in the renal parenchyma. The stainable copper is particularly abundant in the glomerular tuft shown in the left upper portion of the illustration, and in the contents of the tubules. Some is also present in the cytoplasm of the epithelium lining the tubules. Rubeanic acid stain for copper, without counterstain. × 900.

FIG. 2. Greater quantities of copper are contained in the cytoplasm of the tubular epithelium in a mouse sacrificed 24 hours after the intraperitoneal injection of 366 gamma of metal. Rubeanic acid stain for copper, without counterstain. × 420.



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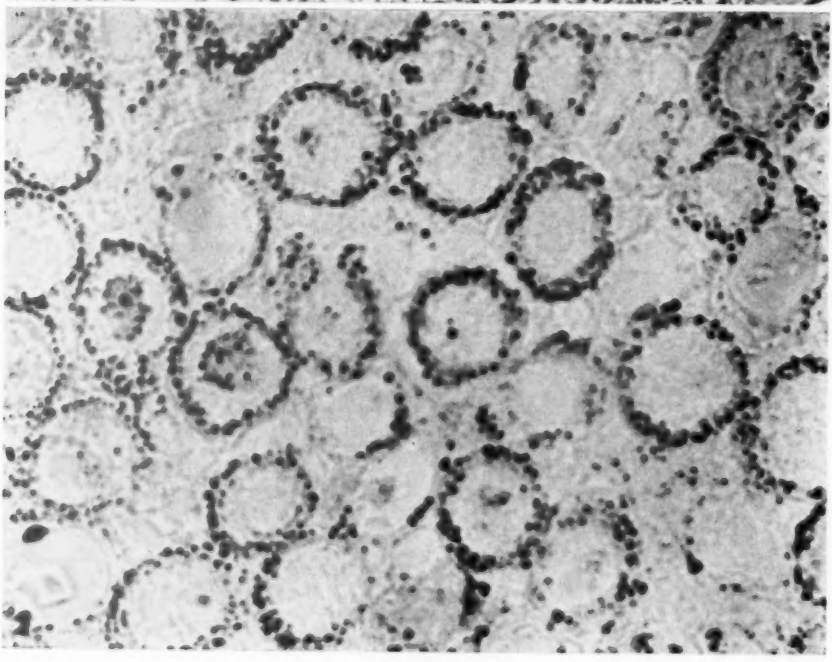

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FIG. 3. There is extensive coagulative necrosis of the proximal convoluted tubular epithelium in a mouse with 366 gamma of copper introduced intraperitoneally 24 hours previously. The renal tissues contain 4.48 gamma of copper per 100 mg. of wet tissue, as shown by chemical analysis. Hematoxylin and eosin stain. × 160.

FIG. 4. Marked calcification is present in the renal tubules of a mouse 72 hours after the intraperitoneal injection of 366 gamma of copper. Von Kossa stain. × 180.

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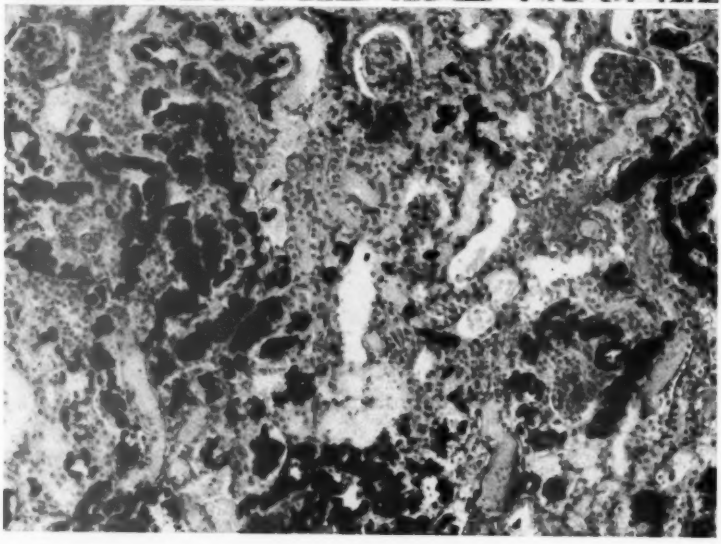

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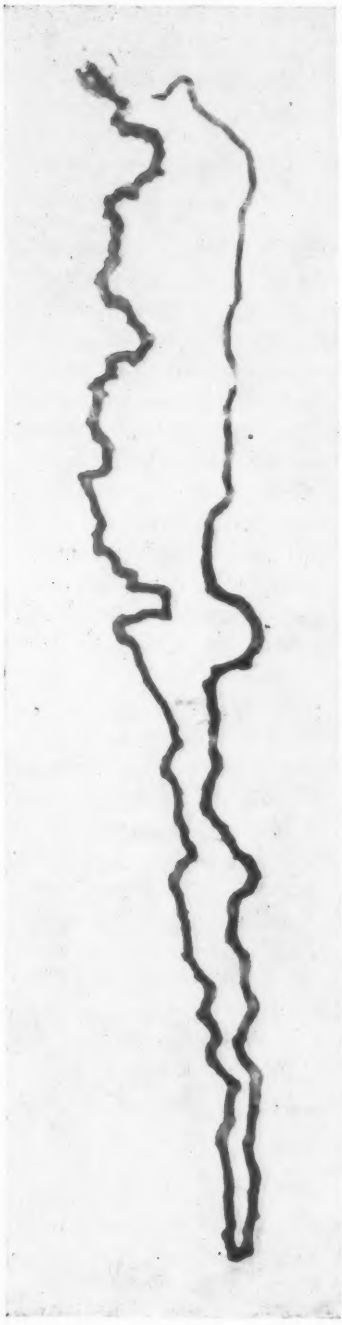
FIG. 5. The normal nephron of a mouse. Mayer's hematoxylin stain. × 38.

FIG. 5A. The normal epithelium of the proximal convoluted tubule forms a regular delicate pattern. Mayer's hematoxylin stain. × 320.

FIG. 6. The proximal convoluted tubule in a mouse 24 hours after the injection of 366 gamma of copper. There are marked, irregular dilatation and extensive degeneration of the epithelium. The basement membrane is swollen. There is a sharp transition at the junction with the nondilated proximal loop of Henle; this segment is lined by intact epithelium. Mayer's hematoxylin stain. × 320.



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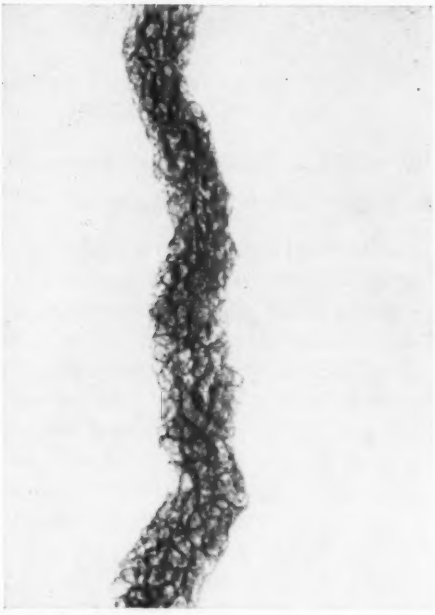

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INVOLUTING AND SCARRED GLOMERULI IN THE KIDNEYS OF INFANTS

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It was Herxheimer [1] who, in 1909, first drew attention to the presence of scarred glomeruli (Fig. 1) in the cortex of kidneys of children dying in the neonatal period. He noted the presence of such glomeruli in a very high proportion (80 per cent) of children he examined. He described the histologic appearance of the scarred glomeruli in detail and, indeed, his description could not be bettered now. He attributed their presence to a developmental imbalance between the epithelial and the mesenchymal parts of the nephron.

The scarred glomeruli show varying features. Minimal lesions consist of an increase in connective tissue around Bowman's capsule and a disappearance of vessels from part of the glomeruli. At the same time there appears to be a reversion of the epithelium of the glomerulus, first to a cuboidal appearance, and later, to columnar cells with palisading. The more severe or later lesion has fibrosis and shrinkage within the glomerulus and a gradual reduction in size of the whole glomerulus and capsule. The final lesion consists of an almost solid circular mass of connective tissue containing a few "epithelioid" cells. The changes in the glomeruli seem to occur at the same time as the scarring about Bowman's capsule.

These scarred glomeruli have aroused relatively little comment, and we have been able to locate only two later studies, that of Schwarz [2] and more recently, Friedman, Grayzel and Lederer.[3] The latter found scarred glomeruli in a small group of infants. At first they considered them evidence of nephritis, but on carrying out a survey of 100 controls and finding the lesions to be present in a high proportion of these also, they suggested the term "congenital glomerulosclerosis." The present paper is concerned with an investigation of the incidence and distribution of scarred glomeruli; this constitutes part of a survey of the postnatal development of the kidney.

MATERIAL AND METHODS

The material comes from two sets of observations. The first represents a series of 200 kidneys analyzed on the basis of the structure of

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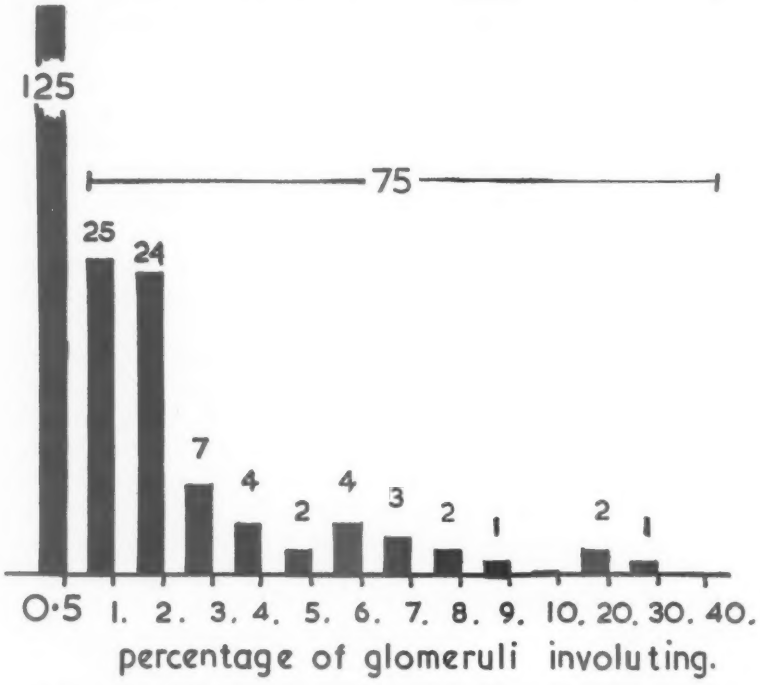
the glomeruli. The second constitutes a separate study of 250 additional kidneys.

For the first study, kidneys were chosen by a system of random numbers from the necropsy records at the Sheffield Children's Hospital.

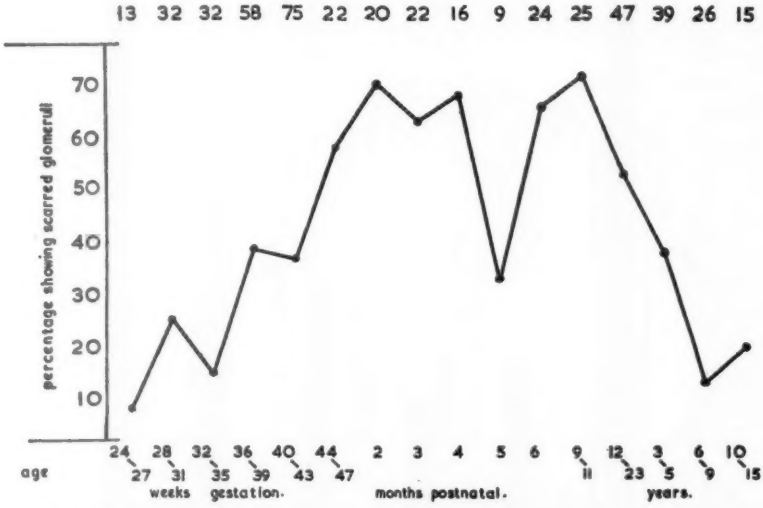


TEXT-FIGURE 1. Histogram showing the frequency distribution of involuting scarred glomeruli from differential counts of 200 glomeruli in each of 200 kidneys.

Kidney sections were procured from blocks that had been cut in true radial fashion and systematic column counts or battlement counts were carried out by using the system commonly applied in differential white cell counts on blood smears. In this case the renal cortex was surveyed from the capsule to the full depth of the cortex. The field was then moved to an adjacent area, and the area surveyed to the capsule, the procedure being repeated. The glomeruli were classified in stages of maturity,[4] and scarred forms were recorded.

Kidneys exhibiting any naked-eye deformity of the renal tract or showing histologic abnormality, e.g., pyelonephritis, were excluded. All the kidneys had been passed previously by one of us (JLE) in routine histologic survey, as being within normal limits. In this survey, approximately 200 glomeruli from each kidney were classified.

During the course of this study, it was noted that the scarred glomeruli were not distributed in a random way throughout the cortex, and a second survey was carried out to record their location. At the same time the presence or absence of abnormally large glomeruli in the juxtamedullary or arcuate zones was noted. In the second series of 250 kidneys, systematic battlement surveys covering between 300 and 500 glomeruli were carried out. Again these kidneys were selected completely arbitrarily from the laboratory files, the ages and diseases of the children not being known to the person examining the sections.



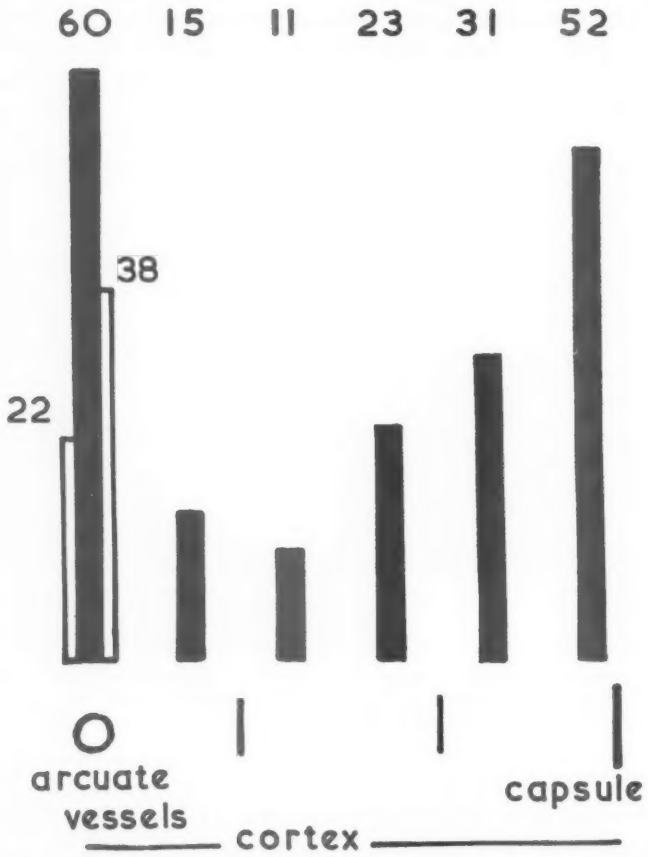
TEXT-FIGURE 2. Graph of the percentage incidence of kidneys showing scarred glomeruli, against age in a series of 475 cases. The figures along the top of the graph are the actual numbers of cases in each age group.

RESULTS

The incidence of scarred glomeruli (Fig. 1) in the 200 kidneys in which differential glomerular counts were carried out is shown in Text-figure 1. The figures represent the percentage of scarred forms found in counts of approximately 200 glomeruli in each kidney. Scarred forms were seen in 75 cases, and not noted in 125. Later inspection of sections from the latter group often revealed scarred forms outside the counted random area—i.e., these 125 represent an incidence of less than 0.5 per cent rather than the absence of scarred forms.

Most of the kidneys with scarred glomeruli showed an incidence of 1 or 2 per cent, but a few contained up to 10 per cent, and 3 kidneys contained the forms in the range of 20 to 30 per cent. The total number

containing scarred forms in a series of 475 children is presented in Text-figure 2, the percentage incidence being related to age. The ages of the children are shown here, as in all the subsequent charts, as age from conception up to 48 weeks (i.e., 2 months after birth, for a 40-week

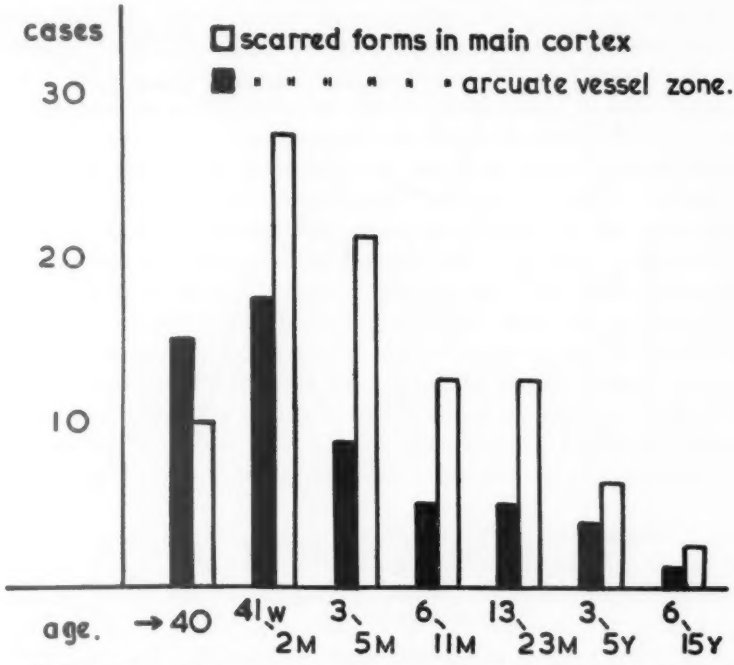


TEXT-FIGURE 3. Diagram showing the distribution pattern of scarred glomeruli in the renal cortex in a series of battlement differential counts of 275 kidneys. The unblocked columns refer to the proportion of the glomeruli on the medullary and cortical sides of the arcuate vessels.

gestation). Over this age they are given as postnatal age. The number of children within each age group is indicated above the graphs. The incidence of scarred glomeruli appeared to increase steadily during the later months of pregnancy and the first postnatal months, and then maintained a steady fall throughout childhood. The lower figures for the

4- to 5-month-old children are probably related to the small number of cases in that age group.

The location of the scarred glomeruli within the cortex is represented diagrammatically in Text-figure 3. For the purpose of this survey, the

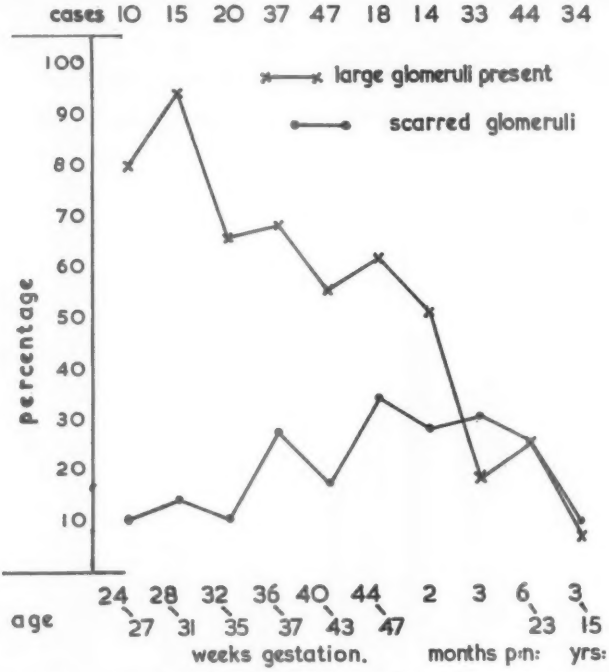


TEXT-FIGURE 4. Diagram showing the separate frequency distribution by age of scarred glomeruli in the arcuate zone and in the remainder of the cortex.

cortex was divided into 6 zones, and the scarred glomeruli in each zone were recorded. The zones were judged more on the number of glomeruli present than on the actual linear depth of the cortex (Fig. 2). This was necessary because in the very young infant, the glomeruli are much more tightly packed at the periphery than at the center, and if the cortex was divided in equal parts, there would be an apparent migration from the capsule toward the medulla in older kidneys. In Text-figure 3, each point does not represent an individual case because each zone in which scarred glomeruli were found was marked—i.e., a kidney showing scarred forms in the arcuate region and immediately beneath the capsule would be charted in both zones.

It is apparent that the scarred forms do not occur in a completely random distribution within the kidney, but are principally located in

two zones, one in the region of the arcuate vessels, and the other in an increasing number as the capsule is approached. This suggests that there are two distinct factors acting in the production of scarred glo-



TEXT-FIGURE 5. Graph showing the frequency distribution by age of large glomeruli and of scarred glomeruli in the juxtamedullary zone.

meruli; one localized about the arcuate vessels and associated with these, and the other directed toward the outer layer of the cortex.

These two zones were therefore analyzed separately, and Text-figure 4 shows the relative proportion of scarred glomeruli in the arcuate zone compared with the rest of the cortex at different ages. Scarred glomeruli in the arcuate region are relatively more common in the very young and immature children than in the older age groups.

It had occurred to us that there might be some relationship between the very large glomeruli found in the juxtamedullary and arcuate region of very young kidneys (Fig. 2) and the presence of large numbers of involuted forms in this area. In Text-figure 5 the two are correlated. The upper graph shows the percentage of kidneys in which large glomeruli were seen in the arcuate region.

These large glomeruli apparently disappear during the first few years

of extra-uterine life, and the fall in numbers appears to be fairly uniform and consistent throughout the first 2 to 3 years. In the newborn the difference in size between the large glomeruli around the arcuate vessels and the rest of the glomeruli is very marked (Fig. 2). In older children this difference is less apparent because of the growth of the other glomeruli. We were aware of the possibility of this fallacy. Thus, a deliberate attempt was made to overcome false assessment, and the ages of the kidneys were not known when examined.

The proportion of kidneys in which scarred glomeruli were found in the juxtamedullary or arcuate zone is shown in Text-figure 5. These glomeruli increase in number during the later stages of intra-uterine life, and appear to be maximal within the first 6 months, falling off rapidly with the disappearance of the large glomeruli in this same zone.

From this survey it would appear that the proportion of scarred glomeruli likely to be found depends upon the age of the kidney examined (Text-fig. 2). We have also gained the impression that an occasional scarred glomerulus would be found in the kidneys of all children if sufficient sections were examined. Also, as can be seen in Text-figure 1, in which the percentages of scarred glomeruli are shown, there is probably a complete gradation from kidneys with isolated individual scarred glomeruli to those showing sufficient scarring to be of pathologic importance.

Our observations correspond reasonably with smaller series in previously published papers, and differences can be accounted for by the numbers of sections examined and differences in age selection. Herxheimer [1] found scarred glomeruli in 38 (88 per cent) of 43 children. Schwarz [2] found them in 45 (56 per cent) of 80 children and infants up to the age of 17 months. When he broke these down into age groups, he found that only 30 per cent of the children under 3 weeks of age showed scarred glomeruli. He did not work out their ages on a gestational basis. Friedman, Grayzel and Lederer [3] found the lesions in all of a selected group of 13 stillborn infants, and later, in an unselected group of 100 children, found 17 examples.

Kampmeier [5] found that in the 48 mm. fetal pig, glomeruli existed near the center of the organ of a size greater than that of the general cortical glomeruli in the 250 mm. piglet. He suggested that these glomeruli were transitory structures.

The distribution of scarred glomeruli within the human renal cortex appears to be in two different areas, suggesting two different populations. One group is associated with the arcuate vessels; these appear early and apparently disappear early in the life of the child, being maximal almost immediately following birth. The second group is found

in increasing numbers as the capsule is approached, and is most prominent during the first two years after birth.

We will discuss the two groups of glomeruli separately. In studying the juxtamedullary zone, attention is quickly drawn to the presence of very large glomeruli in this area. These large glomeruli were noted by Herring.[6] Tsuda,[7] in his measurements of infantile glomeruli, showed that these glomeruli were virtually at adult size in the metanephric kidney in the 4-month fetus, and remained so throughout the whole of fetal life. Such glomeruli can be seen easily (Fig. 2) in the human kidney and frequently give the impression of being almost completely isolated from the rest of the cortex.

We have found that the large juxta-arcuate glomeruli are almost always present in the mid part of intra-uterine life and that they disappear progressively and become uncommon after 6 months following birth (Text-fig. 5). Their disappearance is associated with a high incidence of glomeruli apparently showing progressive fibrosis in the same area. It would seem likely that in these fibrosing glomeruli we are seeing the involution of the large glomeruli and that they are analogous to those which Kampmeier[5] suggested were provisional metanephric structures in the pig and man. Our evidence indicates that there is a single zone of these glomeruli and not a continuous series as Kampmeier[5] postulated.

Concerning the scarred glomeruli in the cortex, we have seen no significant difference between their method of scarring and those near the arcuate vessels. Herxheimer's original description is equally applicable to all of the lesions discussed here. There appears, however, to be an increase in the proportion of scarred glomeruli as the capsule is approached. The glomeruli in the subcapsular area are the most recently formed and are in a state of active proliferation at least until the time of birth[8,9] and in some cases after birth.[4] If the scarred forms in the main cortex were related to age, we would expect to find a larger number of them situated deeply in the cortex than at the periphery. It is the inverse relationship of scarring to maturity that makes the peripheral group of glomeruli seem to have a different cause from that contributing to the isolated large forms near the arcuate vessels. If glomeruli were liable to abnormal development in a random way, those in the deeper layers would be as susceptible to degeneration as those near the cortex. It is possible that this has occurred in the kidneys examined, and that the older abnormal glomeruli have disappeared completely,[10] but this seems to us unlikely. The distribution of lesions suggests some factor acting late in pregnancy or in early postnatal life.

A large number of conditions are known to damage glomeruli in many ways,[11] and metabolic diseases, particularly diabetes,[12] cause intraglo-

merular sclerotic alterations. There are well recognized lesions in the maternal kidneys in toxemia of pregnancy,[13,14] and the infantile kidney could be similarly affected. Von Reuss[15] refers to 4 instances in which children exhibited "nephritis" in the first few days after birth, following eclampsia in the mother.

Without further evidence, any further discussion beyond this point would appear to be pure speculation. In the present series, the kidneys were deliberately selected in a random manner. Perinatal and pregnancy histories are often inadequate, so it would not be justifiable to attempt a complete correlation with clinical features. A further investigation, using only cases with adequate histories, and in which the kidneys will be assessed "blindly," is being attempted.

Summary

A study has been made of the incidence and distribution of scarred glomeruli in the kidneys of a series of 475 children. Scarred glomeruli were found in a range of 10 to 70 per cent of children, depending upon the age of the child. The incidence was highest during the latter part of the first year after birth. The proportion of glomeruli affected was usually in the region of 1 per cent, but occasionally 20 to 30 per cent were affected.

The scarred glomeruli were found in two zones; one near the arcuate vessels and the other near the capsule. It is suggested that the scarred glomeruli in the arcuate vessels represent involution forms of large glomeruli which form very early in this region in intra-uterine life, and possibly represent a transitory renal structure. Peripherally situated scarred glomeruli may be related to disease processes occurring during the later development of the fetus or in the period immediately following birth.

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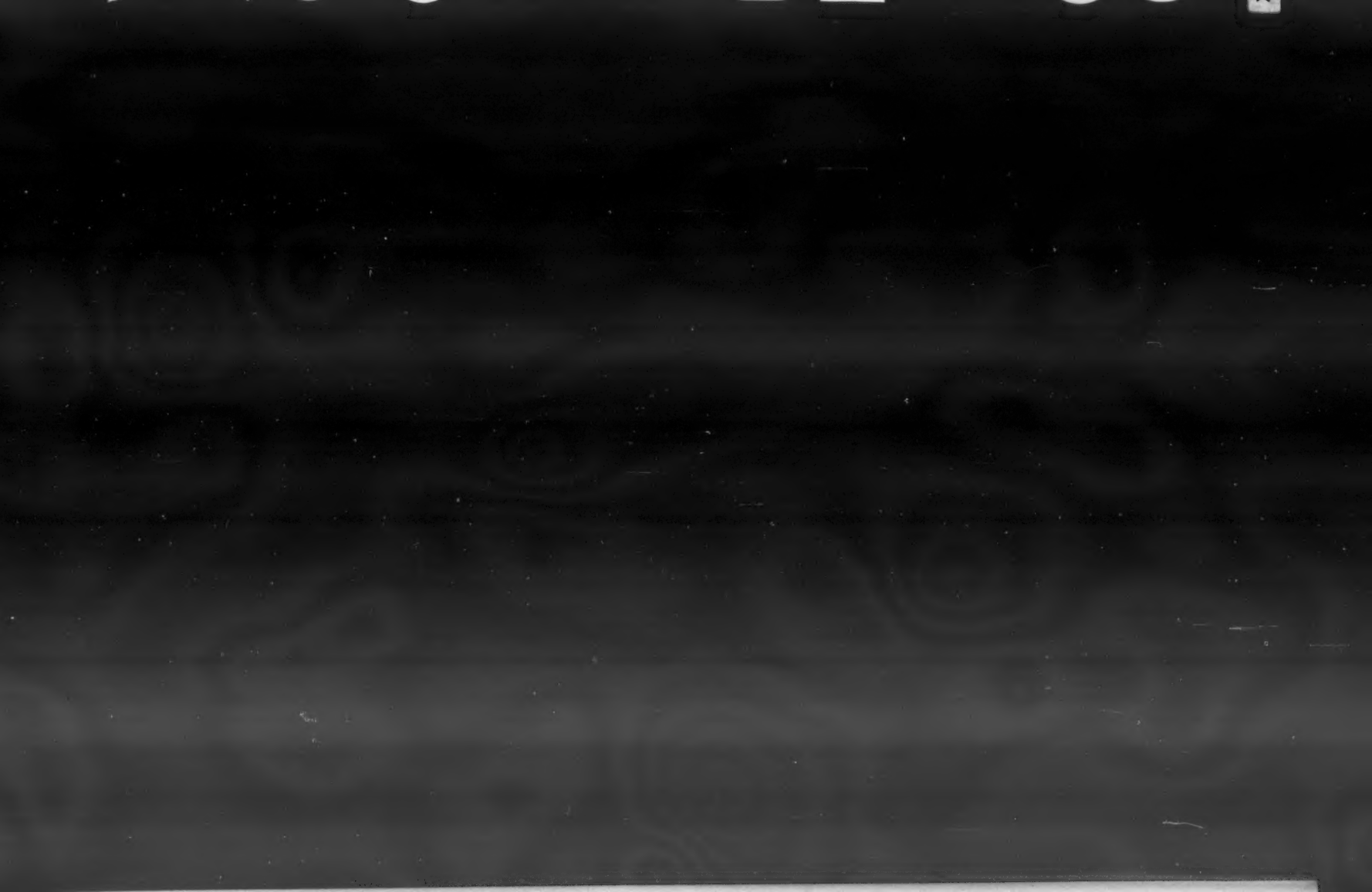
Much of the material used in this study came from necropsies carried out through the courtesy of Dr. A. J. N. Warrack at the City General Hospital, Sheffield. Photographs are by Mr. R. Cousins.

LEGENDS FOR FIGURES

FIGS. 1A and 1B. Two examples of a glomerular tuft being replaced by large fibroblast-like cells; there is an increase in connective tissue around Bowman's capsule. Hematoxylin and eosin stain. × 150.

FIGS. 2A and 2B. The entire depth of the renal cortex in (A) a 36-week infant and (B) a 40-week infant. Primitive glomeruli are seen beneath the capsule (A; arrow). In the deepest part of the cortex adjacent to an arcuate vessel, an isolated large glomerulus can be seen (B; arrow). The circles and lines at the margins of the photographs correspond to the lines shown dividing the cortex in Text-figure 3. Hematoxylin and eosin stain. × 50.



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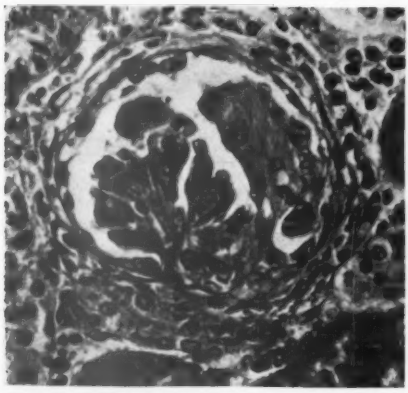
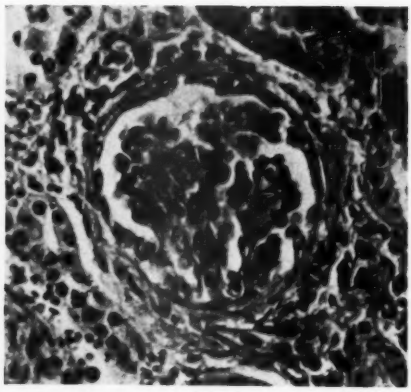
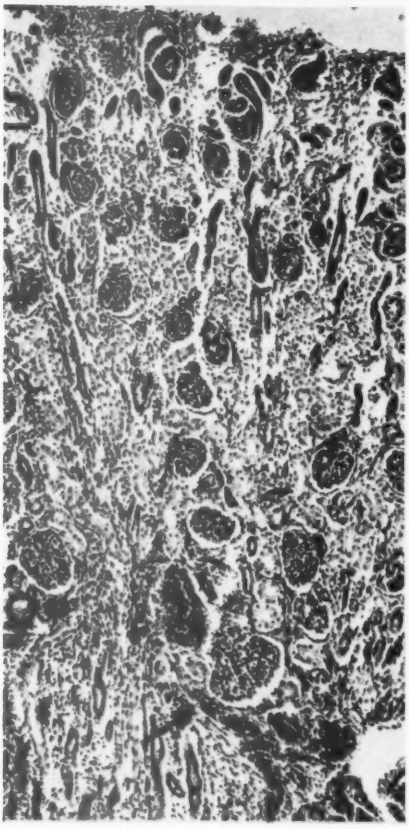
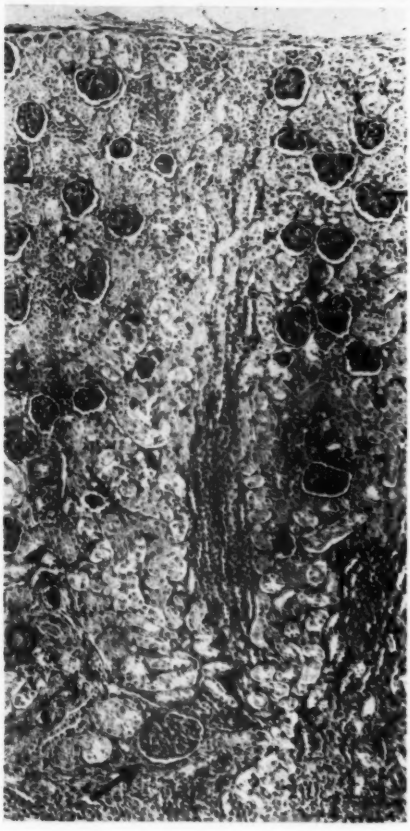




1B

2A





2B

INOCULATION OF HELMINTH EGGS INTO ANIMAL EYES

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From the Department of Public Health and Preventive Medicine and Department of Pathology, Cornell University Medical College, New York, N.Y.

Ocular helminthiasis is a well recognized clinical entity in man.[1] Although cysticercosis, echinococcosis, and sparganosis are known to infect human eyes, ocular lesions are usually attributed to larval nematodes in the stage of active migration.[2–6] In recent years Beaver[6] and his group have produced evidence incriminating the dog ascaris, *Toxocara canis,* as a common migrating worm in any organ, including eyes. In cases of helminthic ophthalmitis, mechanical trauma due to active migration of the parasite contributes substantially to the lesions encountered. It is more difficult to define the nature of host response to the passive presence of a parasitic agent. Therefore, live helminth eggs were inoculated into the eyes of animals, with the hope of evaluating the evolution of ocular response. It was also our intention to determine if the intraocular environment could support the growth of the helminth ova.

Material and Methods

Fresh adult female *Ascaris lumbricoides* var. *suum,*† collected from pigs, were kept alive for several days in a solution containing sodium chloride, potassium chloride, calcium chloride and magnesium sulfate in 7 parts of water, with penicillin and streptomycin added to maintain sterility. To obtain the eggs, the uterus was removed from the worm by aseptic incision and placed in sterile saline in a Petri dish. The portion of the uterus containing mature eggs was dissected away. When pressure was applied to one end of the uterine segment, ova were expelled easily through the other end. This technique allowed the collection of eggs in a relatively unaltered condition without the need to dissolve the uterine walls by chemicals. The ova were washed several times before they were finally placed in 0.5 per cent formalin in a Petri dish and incubated at room temperature for a variable length of time. Fresh eggs as well as those incubated for two weeks were used as inocu-

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* Rockefeller Foundation Fellow; present address: School of Tropical Medicine, Calcutta, India.

† The living worms, *A. lumbricoides,* were obtained through the generosity of Dr. Nathan Entner, New York University, New York, N.Y.

lums. The eggs were washed free of formalin and suspended in normal saline with penicillin and streptomycin added. The suspension (0.05 to 0.1 ml., containing about 20,000 eggs per ml.) was injected into each of the animal eyes with a hypodermic syringe through a 22-gauge needle.

The 15 guinea pigs used in this experiment were divided equally into 5 groups, according to the method, the site of injection, and the time of sacrifice. In group I the inoculum was introduced into the anterior chamber; care was taken to avoid injury to the lens in the right eye, but the lens on the left was deliberately traumatized. The animals in this group were sacrificed one-half hour after the inoculation. Groups II and III received injections in a similar way and were sacrificed after 1 and 2 weeks respectively. This procedure was adopted to determine if lens injury played any role in the course of events, as suggested by Weld and Kean.[7] The guinea pigs in groups IV and V had the eggs introduced into the center of the vitreous body and were sacrificed after 1 and 2 weeks respectively. One guinea pig in each group received the eggs freshly removed from the uteri. The remaining 2 were inoculated with eggs 2 weeks old; these showed a considerable degree of segmentation, but none contained motile larvae inside the egg shell.

Five rabbits were also used. Eggs incubated for 2 weeks were introduced into the anterior chamber of the eyes of 3 rabbits in a manner described for the guinea pigs. One rabbit was sacrificed after one-half hour, another after 48 hours, and the third after 1 week. Inoculation into the vitreous body was made in 2 rabbits; 1 of these was sacrificed after 48 hours and the other after 1 week.

All inoculations were made with the animals under general anesthesia. Sacrifice was accomplished by the introduction of air into the ear veins or by gas. Immediately after sacrifice, the eyes were enucleated and fixed in formalin (10 per cent). They were subsequently embedded in paraffin and cut into 5 to 7 μ sections which were stained with hematoxylin and eosin and with Wright's stain.

Observations

Eggs were found in 8 of 9 guinea pig eyes (groups I, II and III) and in 2 of 3 rabbit eyes when the lens was injured during injection of the inoculum into the anterior chamber. The ova were located mostly in the posterior chamber (behind the iris); in a few sections an isolated egg was encountered inside the lens substance beneath the capsule (Fig. 1) or in the anterior chamber close to the cornea. Among the 9 contralateral eyes of the same guinea pigs, where care was taken to avoid injury to the lens, in only 2 could an occasional egg be found either inside the lens substance (probably because of unintentional injury) or close to

the anterior surface of the iris. No ova were found in the 3 rabbit eyes in which lenticular injury had been avoided.

No appreciable tissue response, immediate or delayed (after 2 weeks), could be detected in or around the sites of lodgment of the eggs in any of 18 guinea pig eyes (Groups I, II and III) and 6 rabbit eyes with eggs implanted in the anterior chamber. Only an assemblage of spherical droplets, presumably lens substance, was found; in most instances these were aggregated around the eggs, especially when the lens was injured (Fig. 2).

Eggs were detected after one week in all 6 guinea pig eyes in group IV, in which the inoculum had been injected into the vitreous body. They were also detected in the rabbits which had been given similar injections —in 2 eyes after 48 hours and in 1 of 2 eyes after 1 week. In all cases they were found to rest innocuously, close to the retina or ciliary body. The presence of eggs did not provoke an appreciable tissue reaction in any of these eyes (Fig. 3).

Eggs examined in microscopic sections showed no further development or growth in any of the sites mentioned. Some had maintained the unicellular stage, or the stage of segmentation seen prior to inoculation. Others were degenerating or disintegrating. Still others were identified only by the presence of empty egg shells.

The eyes of guinea pigs (group V) examined 2 weeks after inoculation into the vitreous body presented a different appearance. Ova could not be found in any of the eyes in this group, but there was a pronounced aggregation of eosinophilic leukocytes in the vitreous humor. In sections of 2 of the eyes, some of the periocular tissues had been included inadvertently. Deeply embedded in this tissue, outside the sclera about half way between the sclerocorneal junction and the posterior pole of the orbit (about the site of the *venae vorticosae*), a number of eggs were found (Fig. 4) surrounded by an intense inflammatory reaction. This was characterized by a heavy accumulation of eosinophils, epithelioid cells and giant cells; in addition there were lymphocytes, plasma cells and some neutrophils. An occasional area of necrosis with a dense peripheral concentration of eosinophils suggested an eosinophilic abscess (Fig. 5). The vascular tunic of the eye, the choroid, was markedly involved in this process. From the root of the ciliary body backward to the point corresponding to the location of the eggs outside the eyes, the entire length of choroid appeared edematous and heavily infiltrated with eosinophils (Fig. 5). A track of eosinophils could be traced from the ova along the adjacent choroidal layer through the root of the ciliary body and into the aggregates of eosinophils within the vitreous body. The retina and sclera were not affected in this process.

Discussion

The lack of an inflammatory reaction to the presence of ova in the anterior and posterior chambers was striking. The manner in which eyes tolerate eggs in these sites is remarkable. It remains to be determined if the unique arrangement in the anterior chamber insures continuous exchange of aqueous humor, thus preventing sufficient concentration of parasitic by-products to excite a tissue reaction. The delayed eosinophil exudate in the vitreous suggests that a prolonged and intimate contact between eggs and tissues in a relatively close environment is necessary to produce significant inflammatory reaction. Should this assumption be true, changes in the anterior chamber in response to a parasitic stimulus are unlikely, unless the tissues are mechanically traumatized to allow a direct and prolonged contact with the parasite.

The localization of ova in the periocular tissue in 2 eyes and their absence from the vitreous body, the site of inoculation, is perplexing. It is, of course, difficult to rule out the possibility of accidental extraocular implantation at the time of inoculation. Nevertheless, the existence of eggs outside both the eyes at nearly identical sites, the simultaneous and uniform disappearance of ova from the vitreous body in all the eyes, and the eosinophil reaction inside the vitreous chamber with the apparent communication via the choroid with the periocular eosinophilic granuloma around the eggs constitute an intriguing pattern. Further study of this phenomenon appears justified.

The failure of development of the eggs, fresh or partially grown, suggests that the environment is unfavorable for this purpose and may be inimical to prolonged survival. This is indicated too by the degeneration and disintegration of some of the ova. Injury to the lens did not seem to contribute in any way to the growth of the ova or to the development of host response. Injury, however, appeared to provide asylum for the eggs and significantly increased the chance of their detection. Failure to recover the eggs in the anterior chamber in the absence of injury to the lens may be attributed to regurgitation along the channel of inoculation during and immediately follwing injection, or to "fall-out" during the processing of tissues for histologic examination.

Summary

Live eggs of *Ascaris lumbricoides* var. ***suum*** were introduced into the anterior chamber of guinea pig and rabbit eyes. These could be detected in histologic sections with a greater frequency when the lens was injured during inoculation. No recognizable tissue alteration was observed up to 2 weeks following inoculation.

Up to one week after inoculation into the vitreous body, no appreciable tissue response was evident, and the eggs remained within the chamber. Two weeks after the inoculation, no ova were detected in this region, but there was a manifest host reaction, characterized by a marked eosinophil response in the vitreous and an eosinophilic choroiditis. Specimens of eyes in which some of the periocular tissues had been inadvertently included revealed the eggs outside the eyes. Here they were surrounded by an intense eosinophilic granulomatous reaction.

No growth or development was observed in the inoculated ova (irrespective of time of examination and area of lodgment). Some of them were found to be dead or dying.

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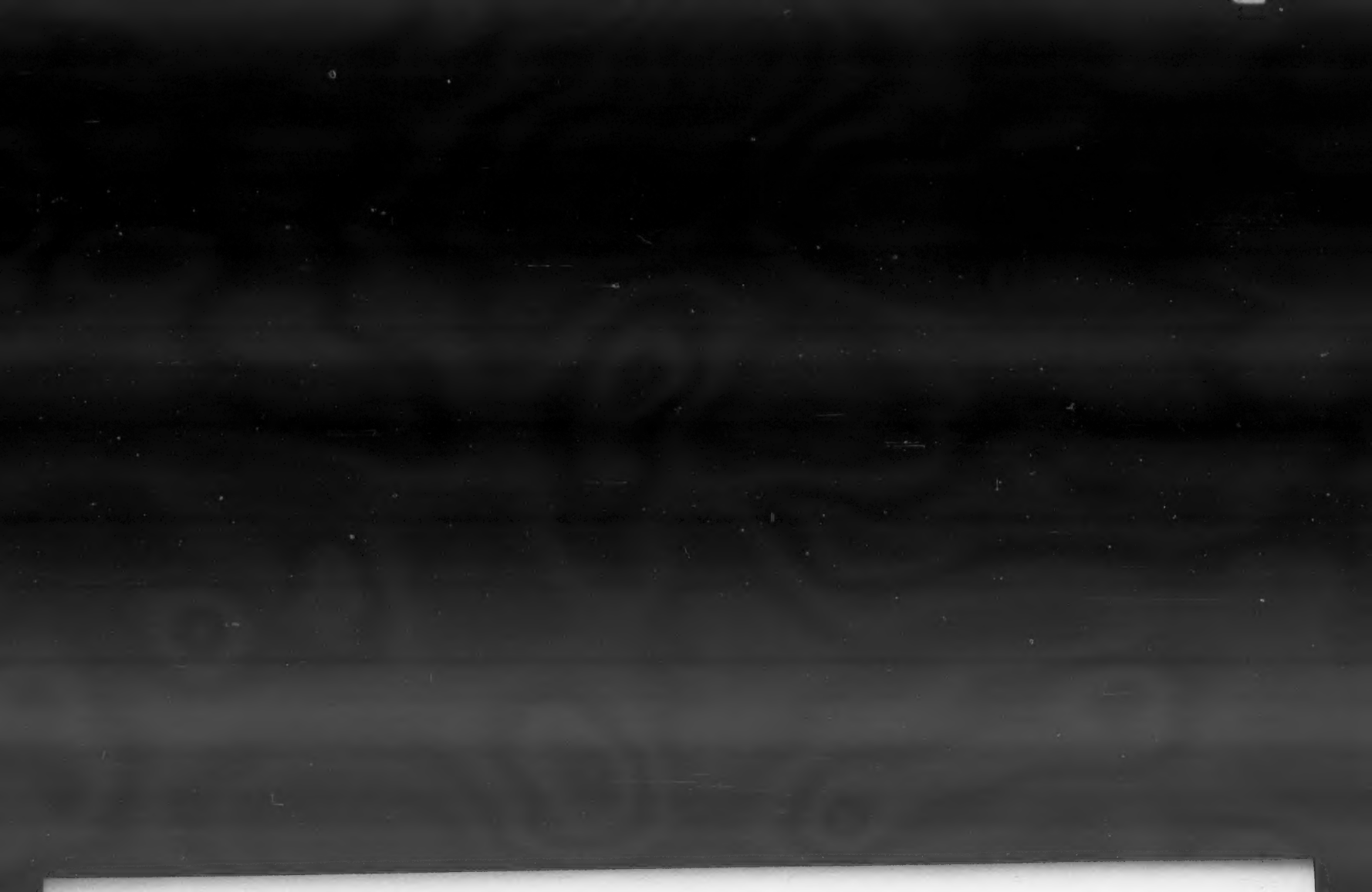
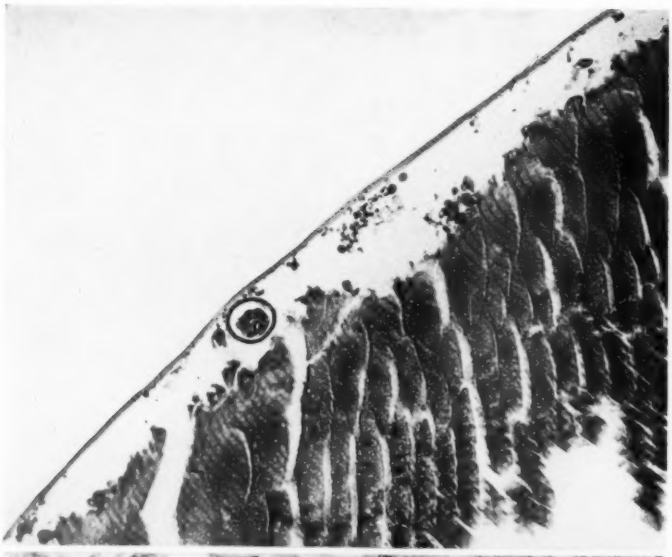
[*Illustrations follow*]

Legends for Figures

Fig. 1. Rabbit's eye, 2 weeks after inoculation. An isolated egg lies inside the lens beneath the capsule. Wright's stain. × 150.

Fig. 2. Rabbit's eye, 2 weeks after inoculation. Ascaris ova appear in the posterior chamber, and there are spherical droplets about them. No cellular reaction is manifest. Wright's stain. × 150.





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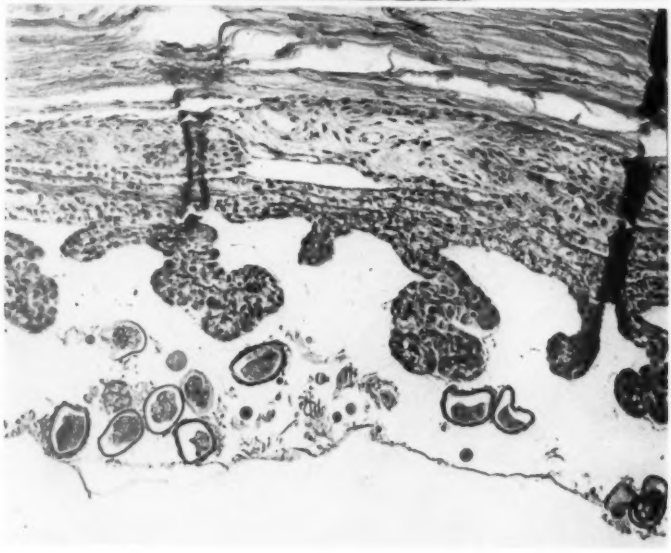

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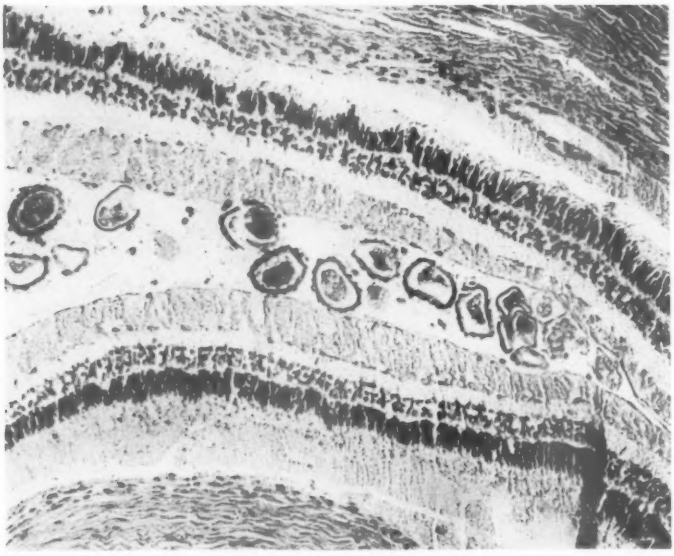

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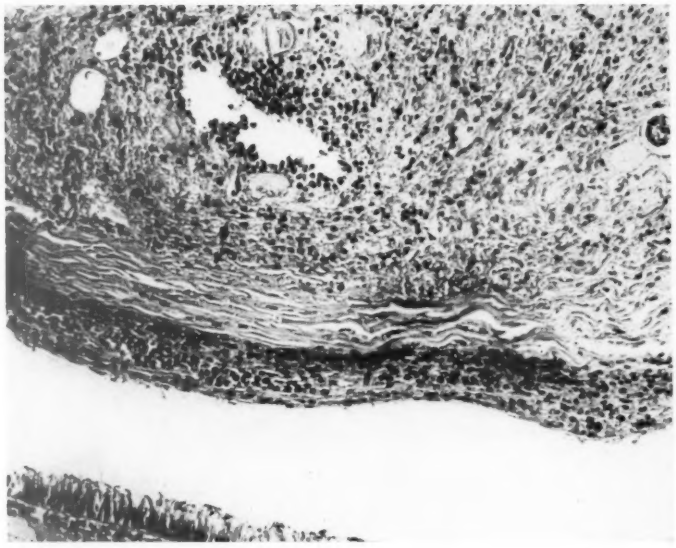

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FIG. 3. Rabbit's eye, 1 week after inoculation. Ascaris eggs appear in the vitreous, but there is no significant tissue reaction. Hematoxylin and eosin stain. × 150.

FIG. 4. Rabbit's eye, 2 weeks after inoculation. Ascaris eggs embedded in the periocular tissue are surrounded by an intense inflammatory reaction with heavy infiltration of eosinophils. Hematoxylin and eosin stain. × 100.

FIG. 5. Rabbit's eye, 2 weeks after inoculation. Ascaris eggs and an eosinophilic abscess appear in the periocular area. The adjoining choroid layer is edematous and heavily infiltrated with eosinophils. Hematoxylin and eosin stain. × 150.

FIFTY-SEVENTH ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Memphis, Tennessee
April 28th, 29th, and 30th, 1960

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Fifty-seventh Annual Meeting

HOTEL PEABODY

Memphis, Tennessee

April 28th, 29th, and 30th, 1960

PRESIDENT SPRUNT IN THE CHAIR

BUSINESS MEETING

The following nominations for elective officers were submitted by the Council:

President	DR. JOHN G. KIDD
Vice-President	DR. D. MURRAY ANGEVINE
Secretary	DR. RUSSELL L. HOLMAN
Treasurer	MAJOR GEN. ELBERT DECOURSEY
Incoming Member of Council	DR. THOMAS D. KINNEY

Additional nominations were called for. None having been offered, it was moved and seconded from the floor that the Secretary be instructed to cast a unanimous ballot for the entire slate.

The President commented on the purposes of the Association and explained briefly the requirements for membership based on long-established custom. He reported the following actions of the Council:

Election of New Members

William R. Adams
Raymond A. Allen
John J. Andujar
William J. Brown
Thomas B. Clarkson, Jr.
Raymond A. Clasen
David L. Coffin
Robert D. Coye
Elemer R. Gabrieli
Jack C. Geer
George G. Glenner
James H. Graham
Henrik A. Hartmann
Earl E. Hellerstein
Frank B. Johnson
Geoffrey Kent
Sidney P. Kent
Paul E. Lacy
Irwin H. Lepow
John R. McDonald
Richard A. MacDonald
Elizabeth A. McGrew
William C. Manion
Geoffrey T. Mann

Elias E. Manuelidis
Mario R. Montenegro
Martin G. Netsky
Edwin T. Nishimura
V. Ramalingaswami
William E. Ribelin
Donald A. Rowley
William F. Scherer
Douglas R. Shanklin
Benjamin H. Spargo
Robert S. Stone
Carl F. Tessmer

William O. Weigle

Re-election of Members of Editorial Board and Assistants to Officers

Assistant Secretary . . . . . Dr. Jack P. Strong
Assistant Treasurer . . . . . Dr. Elson B. Helwig
Editorial Assistant . . . . . Miss Janet E. Smith
Member of the Editorial Board
Dr. Alvin J. Cox, Jr. . . . Term to expire December 1966

With deep regret, the recording of the deaths of:

Earle W. Cauldwell
Alfred E. Cohn
Alfons P. Falkenstein
Lemuel W. Famulener
Leo Loeb
James B. McNaught
Ralph E. Miller
Charles Oberling
John M. Pearce
Louise Pearce
Alex Ragins
Cornelius P. Rhoads
Lawrence H. Sophian
Tom D. Spies

Milton C. Winternitz

The President announced that the next annual meeting (1961) will be held in Chicago, Illinois, in the week of April 24 at the Drake Hotel. The topic for the Symposium will be "Alterations of Fine Structure in Tissues and Cells."

The President further announced that the annual meeting in 1962 will be held in Montreal, Canada.

The results of a polling of the membership concerning the type of program desired and the relative merits of meeting in April or February were reported by the President. A majority of the membership was in favor of continuing single sessions rather than returning to parallel double sessions. A large majority of the membership favored April as the time for the annual meeting.

There being no new business, the Business Meeting adjourned at 2:25 p.m.

Jack P. Strong, *Assistant Secretary*

REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted. It was accompanied by a letter of certification from Ralph Cole, Certified Public Accountant, of Washington, D.C. In condensed form, the Treasurer's report follows:

General Checking Account

Receipts

Balance on hand, January 1, 1959		$ 2,401.49
Transfer of funds, National Bank of Washington, savings account; First & Citizens National Bank, Alexandria, Va., savings account	$ 6,440.75	
Membership dues	13,876.00	
International Academy of Pathology	128.12	
Interest on savings accounts, dividends on shares of stock owned and on shares with savings and loan associations	1,704.56	
		22,149.43
Total receipts		$24,550.92

Disbursements

American Journal of Pathology		$11,076.00	
Secretary's office, clerical	$ 550.00		
Printing, supplies, miscellaneous	2,856.14		
		3,406.14	
Treasurer's office, bonding and auditing	$ 175.00		
Secretarial services	225.00		
Printing, supplies, miscellaneous	444.46		
		844.46	
Miscellaneous			
Intersociety Committee on Pathology Information	$ 500.00		
Intersociety Committee to Increase Research Potential in Pathology	100.00		
International Committee of Pathology	56.42		
Purchases of stocks	7,969.23		
Other	137.51		
		8,763.16	
Total disbursements			$24,089.76
Balance on hand, December 31, 1959			$ 461.16

Investment Inventory

Savings Accounts		
Riggs National Bank, Washington D.C.	$ 4,993.47	
First & Citizens National Bank, Alexandria, Va.	2,151.12	
Olympic Savings & Loan Association, Berwyn, Ill.	5,000.00	
Home Savings & Loan Association, Los Angeles, Calif.	5,000.00	
Mutual Savings & Loan Association, Pasadena, Calif.	5,000.00	
		$22,144.59
Stocks		
209 Shares, Adams Express Company	$ 5,590.63	
100 Shares, United States & Foreign Securities Corporation	3,385.90	
200 Shares, Tri-Continental Corporation	6,261.00	
300 Shares, Blyvooruitzicht Gold Mining Company, Ltd., and		
200 Shares, Consolidated Discovery Yellowknife Mines, Ltd., and		
60 Shares, Kerr-Addison Gold Mines, Ltd.	3,382.22	
200 Shares, Stilfonten Gold Mine	1,200.76	
		19,820.51
Total of investment inventory		$41,965.10
General checking account, Riggs National Bank		461.16
Total assets		$42,426.26

Elbert DeCoursey, *Treasurer*

SCIENTIFIC PROCEEDINGS

PROGRAM

Thursday Morning

1. W. ANTOPOL * AND C. CHRYSSANTHOU, Levy Laboratory, Beth Israel Hospital, New York, N.Y.
   Potentiation of Local Shwartzman Phenomenon by Trypsin.

2. H. E. TAYLOR,* Faculty of Medicine, University of British Columbia, Vancouver, Canada.
   An Immunohistochemical Examination of Granulation Tissue.

3. M. DARIA HAUST AND BENJAMIN LANDING,* Children's Hospital and Children's Hospital Research Foundation, and the University of Cincinnati College of Medicine, Cincinnati, Ohio.
   Histochemical Studies on the Acid Mucopolysaccharide of Hurler's Disease.

4. ROBERT M. O'NEAL * AND W. STANLEY HARTROFT,* Washington University School of Medicine, St. Louis, Mo.
   Production of Atherosclerosis in Rats by Diet.

5. R. W. PRICHARD,* T. B. CLARKSON, H. B. LOFLAND AND M. G. NETSKY, Bowman Gray School of Medicine, Winston-Salem, N.C.
   Effects of Aging and Therapy on Pigeon Atherosclerosis.

6. J. P. STRONG,* J. C. GEER, H. C. MCGILL, JR.,* AND R. L. HOLMAN,* Louisiana State University School of Medicine, New Orleans, La.
   The Histogenesis of Atherosclerosis, Light and Electron Microscopy.

7. BERNARD C. WEXLER,* GEORGE W. KITTINGER AND BENJAMIN F. MILLER, May Institute for Medical Research, the Cincinnati Jewish Hospital; and the University of Cincinnati College of Medicine, Cincinnati, Ohio.
   Mucopolysaccharides, Fibroplasia, and Elastosis in Experimental Arteriosclerosis.

8. ROBERT W. WISSLER,* MERLE S. MOSKOWITZ, RANDOLPH H. HUGHES AND LORRAINE PETRIE, University of Chicago, Chicago, Ill.
   Clinical-Pathological Correlation of the Early Lesions of Arteriosclerosis in Man.

9. RAÚL A. MARCIAL-ROJAS * AND JOSÉ R. CASTRO, University of Puerto Rico School of Dentistry and Dr. I. Gonzalez-Martínez Oncologic Hospital, San Juan, P.R.
   Radiation Injury to Elastic Arteries (a Clinicopathological Study of Eight Cases).

* Asterisks indicate members of The American Association of Pathologists and Bacteriologists. All others appear on the program "by invitation."

10. IRA GORE,* T. NAKASHIMA, T. IMAI AND P. D. WHITE, Harvard Medical School and Harvard School of Public Health, Boston, Mass.; Veterans Administration Hospital, West Roxbury, Mass.; Kyushu University, Fukuoka, Japan; and Massachusetts General Hospital, Boston, Mass.
    Coronary Atherosclerosis and Myocardial Infarction in Kyushu, Japan, and Boston, U.S.A.

11. L. J. MCCORMACK,* D. A. SENHAUSER AND W. L. PROUDFIT, Cleveland Clinic, Cleveland, Ohio.
    Idiopathic Cardiomegaly: Healed Isolated Myocarditis?

12. MAURICE LEV,* Congenital Heart Disease Research and Training Center, Hektoen Institute; Children's Memorial Hospital, and Northwestern University Medical School, Chicago, Ill.
    The Architecture of the Conduction System in Congenital Heart Disease. III. Ventricular Septal Defect.

Thursday Afternoon

1. J. F. A. MCMANUS,* University of Alabama Medical Center, Birmingham, Ala.
    Acid Mucopolysaccharide in the Developing Human Nephron.

2. VARDAMAN M. BUCKALEW AND ASHTON B. MORRISON, University of Pennsylvania School of Medicine, Philadelphia, Pa.
    The Effect of Potassium Deficiency in the Rat with Chronic Renal Insufficiency.

3. JOHN M. CRAIG,* AND F. X. FELLERS, the Children's Medical Center, Harvard Medical School, and the Children's Cancer Research Foundation, Boston, Mass.
    Observations on the Kidney after Phosphate Loading in the Rat; the Effect of pH, Acetazoleamide, Vitamin D Deficiency and Excess, and Parathyroid Extract Injection.

4. L. D. STODDARD,* W. BOELS, E. W. CHICK AND H. C. SUSSMAN, Medical College of Georgia, Augusta, Ga.
    Hypotoxic Tubular Necrosis of the Kidney, Experimentally Produced in the Rabbit by Temporary Unilateral Renal Ischemia.

5. J. S. HOWE,* M. F. WATT, F. E. HATCH, JR., AND A. E. PARRISH, Veterans Administration and George Washington University Hospitals, Washington, D.C.
    Histogenesis of Diabetic Glomerulosclerosis Studied by Needle Biopsy of the Kidney.

6. PAUL H. GUTTMAN * AND HENRY I. KOHN, Cancer Research Institute and the Radiological Laboratory, University of California Medical Center, San Francisco, Calif.
    Progressive Intercapillary Glomerulosclerosis in the Mouse, Rat, and Chinese Hamster, Associated with Aging and X-ray Exposure.

7. HENRY Z. MOVAT * AND JAN W. STEINER, University of Toronto, Toronto, Canada.
   The Progression of Lipoid Nephrosis to Chronic Glomerulonephritis—Electron Microscopic Studies.

8. J. J. VAZQUEZ,* J. D. FELDMAN,* F. J. DIXON * AND W. O. WEIGLE, University of Pittsburgh School of Medicine, Pittsburgh, Pa.
   The Morphology and Immunology of Chronic Irreversible Glomerulonephritis in Rabbits.

9. WILLY MAUTNER, ALBERT ALTCHECK, EDITH GRISHMAN * AND JACOB CHURG,* the Mount Sinai Hospital, New York, N.Y.
   Renal Biopsies in Pre-eclampsia—Electron and Light Microscopic Studies of Glomerular Changes.

10. CONRAD L. PIRANI,* ROBERT LANNIGAN, VICTOR E. POLLAK AND ROBERT C. MUEHRCKE, University of Illinois College of Medicine, Chicago, Ill.
    Electron Microscopic Studies of the Renal Glomerulus in Toxemia of Pregnancy (Pre-eclampsia).

11. BORIS GUEFT * AND JOHN GHIDONI, Albert Einstein College of Medicine, New York, N.Y.
    The Site of Formation and Ultrastructure of Amyloid.

12. WILLIAM F. MCCORMICK, University of Tennessee and the City of Memphis Hospitals, Memphis, Tenn.
    Pathology of Sickle Cell Trait.

Friday Morning

SYMPOSIUM ON GENETIC FACTORS IN DISEASE

Referee (by invitation of the Council): C. Nash Herndon

1. M. A. FERGUSON-SMITH, Western Infirmary, Glasgow, Scotland.
   Chromosomal Abnormalities in Man: Recent Developments.

2. K. PATAU, E. THERMAN AND D. W. SMITH, University of Wisconsin, Madison, Wis.
   Abnormal Chromosome Constitutions in Man as Cause of Multiple Congenital Anomalies.

3. WILLIAM H. STERNBERG * AND H. WARNER KLOEPFER, Tulane University Medical School, New Orleans, La.
   Genetic and Pathologic Study of Simulant Females (Testicular Feminization Syndrome).

4. VICTOR A. MCKUSICK, Johns Hopkins University School of Medicine, Baltimore, Md.
   The Basic Defect in Certain Heritable Disorders of Connective Tissue.

5. STANLEY M. ARONSON * AND BRUNO W. VOLK,* Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, and State University of New York, Downstate Medical Center, Brooklyn, and Albert Einstein College of Medicine, New York, N.Y.
   Certain Genetic, Pathologic and Biochemical Characteristics of the Infantile Nervous System Lipidoses.

6. ROBERT FIENBERG,* Beverly Hospital, Beverly, Mass.
   Perinatal Idiopathic Hemochromatosis: Giant Cell Hepatitis Interpreted as an Inborn Error of Metabolism.

7. JOHN B. GRAHAM,* CARL J. WITKOP, JR., AND CLEMM SHANKLE, University of North Carolina, Chapel Hill, N.C., and the National Institute of Dental Research, Bethesda, Md.
   Hereditary Benign Intraepithelial Dyskeratosis: a "New" Mucous Membrane Syndrome.

8. HENRY GERSHOWITZ, University of Michigan, Ann Arbor, Mich.
   The Relation of Blood Groups to Leukemia, Nephrosis-Nephritis, Congenital Heart Defects and Rheumatic Fever; Sibship Analysis.

9. GEORGE YERGANIAN, MORRIS N. GREEN AND HANS MEIER,* Children's Cancer Research Foundation, Harvard Medical School, and the Children's Hospital, Boston, Mass.
   Spontaneous Hereditary Diabetes Mellitus in the Chinese Hamster: Pathologic, Biochemical, and Genetic Findings.

10. C. NASH HERNDON (Referee †), Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N.C.
    Frontiers Common to Pathology and Genetics.

† By special invitation of the Council.

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Friday Afternoon

1. I. J. PINCUS, I. N. DUBIN,* FLORA PASCASIO AND MARY M. PORTER, Woman's Medical College of Pennsylvania, Philadelphia, Pa.
   Studies on Obstructive Jaundice in the Dog.

2. C. T. ASHWORTH * AND E. SANDERS, the University of Texas Southwestern Medical School, Dallas, Texas.
   Anatomic Pathway of Bile Formation.

3. J. W. GRISHAM, Washington University School of Medicine, St. Louis, Mo.
   Changes in the Fine Structure of the Hepatic Cell in the Choline-deficient Rat.

4. HANS F. SMETANA,* G. GORDON HADLEY,* AND M. V. SIRSAT, Patel Institute, Delhi University, Delhi, India; Armed Forces Institute of Pathology, Washington, D.C.; Christian Medical College and Hospital, Vellore, South India; College of Medical Evangelists, Redlands, Calif., and Indian Cancer Research Center, Bombay, India.
   The Histogenesis of Infantile Cirrhosis.

5. EMANUEL RUBIN, STEFAN KRUS AND HANS POPPER,* the Mount Sinai Hospital, New York, N.Y.
   Histogenesis of Postnecrotic Cirrhosis in Alcoholics.

6. SYDNEY S. LAZARUS * AND BRUNO W. VOLK,* Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn, N.Y., and Albert Einstein College of Medicine, New York, N.Y.
   Rabbit Pancreas in Protein Malnutrition (Experimental Kwashiorkor) and after Cortisone Administration.

7. NATHAN, KAUFMAN,* THOMAS D. KINNEY,* JANIS V. KLAVINS,* ROGER W. MARSTERS AND CHING Y. TSENG, Western Reserve University School of Medicine and Cleveland Metropolitan General Hospital, Cleveland, Ohio.
   Plasma Amylase Values in Animals with Ethionine Induced Pancreatic Damage.

8. STANLEY F. PATTEN, JR., AND THOMAS SFILIGOJ, Western Reserve University, Cleveland, Ohio.
   The Action of Cysteine on the Intestinal Epithelium Following Nitrogen Mustard Administration as Demonstrated by Autoradiographs.

9. M. STRAUB AND J. SCHWARZ,* Jewish Hospital and Cincinnati General Hospital, Cincinnati, Ohio.
   Spontaneous Primary Histoplasmosis in Dogs in Cincinnati (Ohio).

10. EUGENE J. JOSEFIAK, J. H. SMITH FOUSHEE AND WILLIAM J. REEVES, Bowman Gray School of Medicine and North Carolina Baptist Hospital, Winston-Salem, N.C.
    Aspergillus Infections of the Lungs.

11. FRANZ VON LICHTENBERG,* Peter Bent Brigham Hospital, Boston, Mass.
    Host Reaction to Purified Eggs of *S. mansoni*. I. Pulmonary Granuloma Formation in Unsensitized Mice.

12. W. S. GILMER, JR.,* AND E. J. EICHLER, University of Tennessee Medical Units and the Campbell Clinic, Memphis, Tenn.
    Histogenesis of Pulmonary Perivascular Cell Collections.

13. JEROME KLEINERMAN,* G. W. WRIGHT AND ELINOR ZORN, St. Luke's Hospital, Cleveland, Ohio.
    The Elastin and Collagen Content of Normal and Emphysematous Human Lungs.

Saturday Morning

1. RICHARD B. MARSHALL AND ROBERT C. HORN, JR.,* Henry Ford Hospital, Detroit, Mich.
   Nonchromaffin Paraganglioma—a Comparative Study.

2. G. B. PIERCE, JR.,* AND F. J. DIXON, JR.,* University of Pittsburgh School of Medicine, Pittsburgh, Pa.
   Embryonic Nature of Neoplastic "Embryoid Bodies" of a Teratocarcinoma.

3. Donald W. King * and Festus O. Adebonojo, Yale University School of Medicine, New Haven, Conn.
   Effect of Anaerobiosis on the Enzymatic Pattern of Strain L Cells.

4. P. O'B. Montgomery,* University of Texas Southwestern Medical School, Dallas, Texas.
   Basic Reactions of Living Protoplasm to Ultraviolet Irradiation.

5. Wallace H. Clark, Jr., Tulane University School of Medicine, New Orleans, La.
   Nuclear "Blebs"—Structures Suggesting the Transfer of Nuclear Material into the Cytoplasm.

6. Bill M. Nelson and C. Harold Steffee, Orins Medical Division, Oak Ridge, Tenn.
   Autopsy Findings after Treatment of Leukemia with Total-body Irradiation and Homologous Marrow Infusions.

7. George D. Sorenson, Washington University School of Medicine, St. Louis, Mo.
   Electron Microscopic Observations of Bone Marrow from Patients with Hypochromic-Hypersideremic Anemia.

8. Richard B. Cohen, Massachusetts General Hospital and Harvard Medical School, Boston, Mass.
   Adenomatous Regeneration of the Cortical Remnant of Bilaterally Hyperplastic Adrenal Glands Subtotally Resected for Cushing's Syndrome.

9. Herbert C. Stoerk,* Merck Institute for Therapeutic Research, Rahway, N.J.
   Degenerative Changes in Epiphyseal Chondrocytes of Parathyroidectomized Rats.

10. C. Alexander Hellwig * and P. N. Wilkinson, Hertzler Research Foundation, Halstead, Kans.
    Experimental Production of Chronic Thyroiditis.

11. Robert P. Bolande,* Western Reserve University, Cleveland, Ohio.
    Inclusion-bearing Cells in Urine Following Vaccination with Attenuated Live Measles Virus.

12. William J. Cheatham, Jerome H. Abramson and John L. Shapiro,* Vanderbilt University School of Medicine, Nashville, Tenn., and Grady Memorial Hospital, Atlanta, Ga.
    Isolation of Adenovirus from a Fatal Case of Interstitial Pneumonia with Nuclear Inclusions.

13. Stephen S. Sternberg,* Frederick S. Philips, and Alice P. Cronin, Sloan-Kettering Institute for Cancer Research, and Memorial Hospital, New York, N.Y.
    Biological Actions of Dithizone, with Special Reference to Prostatic Necrosis and Atrophy.

READ BY TITLE

1. W. ANTOPOL,* H. ALBAUM, S. SLAPIKOFF, B. KABAKOW, L. SUSSMAN, G. BLINICK AND L. GINZBURG, Levy Laboratory, Beth Israel Hospital, New York, N.Y.
   Serum Enzyme Changes after Treatment of Malignant Tumors.

2. WALTER R. BENSON,* University of North Carolina School of Medicine, Chapel Hill, N.C.
   The Ability of the Ethionine-injured, Regenerated Pancreas to Reconstitute Secretory Granules.

3. WILLIAM M. BERTON, University of Tennessee, Memphis, Tenn.
   Correlative Studies of the Vaginal Fluid Microflora of the Young *Macaca mulatta*.

4. EDWIN R. FISHER,* University of Pittsburgh and Veterans Administration Hospital, Pittsburgh, Pa.
   Augmentation of Cholesterol Atherosclerosis in Hypertensive Diabetic Rabbits.

5. G. M. GOLDBERG, A. I. RUBENSTONE, AND O. SAPHIR,* Michael Reese Hospital, Chicago, Ill.
   Mononuclear Leukemias and the Lymphatics of the Spleen.

6. IRA GORE,* ANNE BLENKE AND DANIEL COLLINS, Harvard Medical School, Peter Bent Brigham Hospital, and Veterans Administration Hospital, Boston, Mass.
   Pulmonary Arterial Bands Resulting from Pulmonary Embolization.

7. M. DARIA HAUST AND ROBERT H. MORE,* Queen's University, Kingston, Ont., Canada.
   Atherogenesis and Its Relation to Blood Proteins.

8. CORNELIA HOCH-LIGETI,* University of Virginia School of Medicine, Charlottesville, Va.
   Effect of 3:5:3′ Triiodothyronine (T_3) on the Nucleic Acid and Succinic Dehydrogenase Contents of Organs of Enthyroid Rats.

9. RUSSELL S. JONES,* CLAUDE R. THOMAS AND E. VIRGIL HOWELL, University of Utah College of Medicine, Salt Lake City, Utah.
   Adrenal Cortical Function and Induced Regression of the Murphy-Strum Lymphosarcoma.

10. JOHN M. KISSANE AND ARA CHALVARDJIAN, Washington University School of Medicine, St. Louis, Mo.
    Quantitative Histochemistry of the Nephron and Juxtaglomerular Apparatus.

11. SIDNEY D. KOBERNICK,* Sinai Hospital of Detroit, Detroit, Mich.
    The Inhibition by Exercise of Spontaneous Aortic Lesions in Rabbits and Its Significance in Atherosclerosis.

12. Sydney S. Lazarus * and Bruno W. Volk,* Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn, and Albert Einstein College of Medicine, New York, N.Y.
    Toxic Nephrosis in Rabbits after the Oral Hypoglycemic Drug Phenformin (DBI).

13. Kyu Taik Lee, Sang Chul Nam and Sung Bae Kin, Kyungpook University School of Medicine, Taegu, Korea.
    Diet, Serum Lipids and Coronary Heart Disease in Koreans.

14. Kyu Taik Lee, Dong Nack Kim and Yeun Sick Kwack, Kyungpook University School of Medicine, Taegu, Korea.
    Diets, Serum Lipid Levels and Electrocardiograms of 129 Korean Buddhist Priests, Pure Vegetarian.

15. Searle McMurry, O. Randolph Batson and John L. Shapiro,* Vanderbilt University School of Medicine, Nashville, Tenn.
    Fatal Unexplained Granulomatous Disease of Childhood.

16. J. W. Mitchener and W. G. Rice,* Medical College of Georgia, Augusta, Ga.
    Histochemical Evidence of 5-Hydroxytryptamine in a Dog Mast Cell Tumor.

17. E. E. Muirhead,* Wayne State University College of Medicine, Detroit, Mich.
    Autoexplanation of Renal Tissue to Peritoneum and Lungs.

18. Hans H. Naumann * and Joseph M. Young,* Kennedy Veterans Administration Medical Teaching Group Hospital, Memphis, Tenn.
    Use of Vitreous Body for Postmortem Chemistry in Cases of Diabetes and Uremia.

19. H. E. Nieburgs, the Mount Sinai Hospital, New York N.Y.
    Objective Cell Interpretation in Cytologic and Histologic Specimens.

20. W. L. Past and C. D. Eversole, University of Louisville School of Medicine, Louisville, Ky.
    The Histologic Demonstration of Iron in Bones, Following EDTA Decalcification.

21. Bjarne Pearson * and Fred Grose, Wayne State University and Detroit Institute of Cancer Research, Detroit, Mich.
    Quantitative Cytologic Study of Anhydrous Mass in Regenerating Liver.

22. E. A. Porta, J. W. Grisham and W. S. Hartroft,* Washington University School of Medicine, St. Louis, Mo.
    An Electron Microscopic Study of the Cytogenesis of Ductular Cells.

23. Harold E. Snyder and John L. Shapiro,* Vanderbilt University School of Medicine, Nashville, Tenn.
    Cholesterol Embolism in Human and Experimental Animals.

24. Minoru Suzuki and Robert M. O'Neal,* Washington University School of Medicine, St. Louis, Mo.
    Cholesterol Esterification in Rats with an Extreme Modification of "Cholesterol-Fatty" Liver.

25. BRUNO W. VOLK * AND SYDNEY S. LAZARUS,* Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn, and Albert Einstein College of Medicine, New York, N.Y.
    The Diabetic Pancreas: a Morphologic Re-evaluation Utilizing Refined Histologic Techniques.

26. C. A. WAGENVOORT, State University, Leyden, The Netherlands.
    A Method to Distinguish Between Vasoconstriction and Medial Hypertrophy in Pulmonary Arterial Branches.

27. DOUGLAS WAUGH * AND MANUEL J. PEARL, Queen's University and Hotel Dieu Hospital, Kingston, Ont., Canada.
    Serotonin-induced Acute Nephrosis and Renal Cortical Necrosis in Rats. A Morphological Study with Pregnancy Correlations.

28. JOHN P. WYATT * AND HERBERT SWEET, St. Louis University School of Medicine, St. Louis, Mo.
    On the Postmortem Structural and Functional Alterations in Generalized Panlobular Emphysema.

29. H. WARNER KLOEPFER AND JOHN MOOSSY, Tulane University School of Medicine and Louisiana State University School of Medicine, New Orleans, La.
    Pathological and Genetic Study of a Type of Progressive Dementia with Oligophrenia and Erythema.

INDEX OF SUBJECTS

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